3. HEALTH EFFECTS

3.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of mustard gas. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

The term mustard gas is used to refer to a variety of compounds. In its most common sense, it refers to sulfur mustard (bis[2-chloroethyl]sulfide). This is the compound discussed in this profile. The term mustard gas has also been occasionally applied to nitrogen mustards, which have not been reported at NPL sites and are not evaluated in this profile. Mustard gas is a clear, colorless, oily liquid. As a warfare or terrorist agent, mustard gas may have been dispersed by spraying or by explosive blasts producing a vapor, aerosol, and/or liquid droplets. Persons involved in the manufacture, transport, or disposal of mustard gas may also be exposed occupationally. Mustard gas corrodes and weakens containers and may be dispersed in water, cleaning solvents, or by evaporation in air. Mustard gas is slightly soluble in water, but both the liquid and vapor forms are readily soluble in alcohol, gasoline, kerosene, oils, fats, and organic solvents. Mustard gas is environmentally persistent. Evaporation in air increases with increasing temperatures, but at temperature below 14 EC, it freezes and remains in active form. Both liquid and vapor forms readily penetrate ordinary clothing. The effects of mustard gas poisoning may be local, systemic, or both, depending on environmental conditions, exposed organs, and extent and duration of exposure. Because of the high lipid solubility, mustard gas quickly penetrates the lipid cell membrane. Although mustard gas may be lethal, it is more likely to cause extensive incapacitating injuries to the eyes, skin, and respiratory tract of exposed persons. Alkylation reactions of mustard gas with tissue are rapid and irreversible; however, cutananeous lesions do not become apparent for one to several hours after exposure. Burns caused by mustard gas are typically severe and require long healing periods.

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure (inhalation, oral, and dermal) and then by health effect (death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects). These data are discussed in terms of three exposure periods: acute (14 days or less), intermediate (15–364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELS have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user's perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAELs) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

Estimates of exposure levels posing minimal risk to humans (Minimal Risk Levels or MRLs) have been made for mustard gas. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

A User's Guide has been provided at the end of this profile (see Appendix B). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

3.2.1 Inhalation Exposure

While mustard gas is described as smelling like mustard, horseradish, garlic, or onions, it can be difficult to smell and may not be recognized by the general population. Due to the delayed symptoms and difficulties associated with detection by smell, individuals may not know that they are being exposed, and consequently, appropriate actions may not be taken. The odor threshold for mustard gas is 0.6 mg/m^3 (0.0006 mg/L) (SBCCOM 1999). In humans, an ICt_{50} (estimated concentration-exposure time period product incapacitating to 50% of exposed individuals) for inhalation exposure is 1,500 mg-minute/m^3 (SBCCOM 1999).

3.2.1.1 Death

Human deaths associated with mustard gas exposure occurred during World War I (an estimated 28,000 deaths), during the Italian attack on the Ethiopians in 1936, and during the Iran-Iraq War in 1980–1988. Battlefield air concentrations of mustard gas vapor during attacks in World War I were estimated in the range 19–33 mg/m^3 (Solberg et al. 1997). During chemical warfare, exposure to mustard gas generally occurred by multiple routes. Mustard gas can cause death in several ways, and with multiple routes, it is often difficult to determine the relative importance of local and systemic effects in causing death. Death is often accompanied by heavy and painful coughing, vomiting, burning eyes, and shock. Deaths have occurred immediately following exposure in the battlefield, most likely due to acute chemical-induced pulmonary edema (Freitag et al. 1991). Mustard gas has caused death within a few hours of exposure by inducing shock in victims of the World War II Bari Harbor incident and in civilians who accidentally recovered unspent World War I mustard gas shells (Alexander 1947; Papirmeister et al. 1991). Deaths beyond the second day after the Bari incident were attributed to decreased leukocyte counts, which reached levels below 100 cell/cm^3 (Dacre and Goldman 1996). While mustard gas was not used during World War II, cargo vessels in the harbor of Bari, Italy, carrying mustard gas and explosive munitions were bombed by German planes. In the resulting explosion, mustard gas was released in to the air and water, exposing survivors to mustard gas vapor and to a mixture of mustard gas in oil. Deaths, which occurred in 1–4% of the soldiers exposed during World War I, were largely due to secondary respiratory infections. Accidental death of a family of two adults and two children occurred in 1919 in Salaise, France after exposure to mustard gas, which evaporated from a leaking can of mustard gas-contaminated alcohol that was being stored in the house (Dacre and Goldman 1996).

One death among 14 children (9 boys, aged 9 months to 14 years; 5 girls, aged 13 months to 9 years) admitted to a hospital in Iran 18–24 hours following exposure to mustard gas from air bombs during the Iran-Iraq War was reported (Momeni and Aminjavaheri 1994). The 13-month-old girl developed pancytopenia and respiratory failure, and died 8 days after exposure. Deaths have also occurred from delayed responses (DOA 1988; Lohs 1975; Somani and Babu 1989). Further information on delayed death due to inhalation of mustard gas by humans is discussed below in the sections on respiratory effects in Section 3.2.1.2 and on cancer in Section 3.2.1.7. Lethal doses for humans have been reported, but these data cannot be considered to be reliable because all elements of exposure were not clearly described (Frank 1967; Rosenblatt et al. 1975).

Rabbits and monkeys that had undergone tracheal cannulation were exposed to nominal chamber concentrations of mustard gas ranging from 30 to 350 mg/m^3 (5–54 ppm) (Cameron et al. 1946). While incidence data were not provided, Cameron et al. (1946) reported that mustard gas vapor produced lethal effects in rabbits and monkeys in the absence of lung damage, indicating that lethal doses may be absorbed through the mucous membrane of the nose.

In animals, no deaths attributable to mustard gas were noted in mice, rats, guinea pigs, rabbits, or dogs exposed for up to 1 year to 0.1 mg/m^3 (0.015 ppm) (McNamara et al. 1975). Complete experimental details were not provided in this report from the Army.

3.2.1.2 Systemic Effects

The highest NOAEL and all LOAEL values for each study for systemic effects in each species are recorded in Table 3-1 and plotted in Figure 3-1.

Respiratory Effects. Respiratory effects have been found in humans following acute and chronic exposures. Under warm environmental conditions, the respiratory effects of mustard gas were observed to be increased. While durations and levels are not known, toxicologic analyses indicated that some soldiers were exposed to mustard gas during the Iran-Iraq War (1980–1988) (Momeni et al. 1992). Soldiers reported shortness of breath and early respiratory manifestations including hemorrhagic inflammation of the tracheobronchial mucosa accompanied by severe erosions or membranous lesions. Some exposed soldiers became temporarily aphonic due to an acid-like burning sensation of the vocal cords. Brush smears from the trachea and main bronchi showed irregular ciliary beating with lower mean frequencies (9 Hz on average) compared to the normal 10–20 Hz range. In children, cough was the first respiratory symptom (Momeni and Aminjavaheri 1994). Secondary complications consisted of extensive stenosis of sections or the entire tracheobronchial tree, suppurative bronchitis, and chronic respiratory infections with *Staphylococcus aureus*, *Hemophilus influenzae*, and *Pseudomonas aeruginosa* resistant to appropriate antibiotic therapy. Scars, ulcers, strictures, and nonspecific fibrous granulation developed in central airways after a delay up to 15 months. Progressive deterioration of lung compliance and gas exchange with resulting hypoxemia and hypercapnia, were common with injury. Chronic respiratory complaints included shortness of breath, chest tightness, cough, sneezing, rhinorrhea, and sore throats (Momeni et al. 1992; Somani and Babu 1989). Long-term or delayed effects included central airway

Table 3-1. Levels of Significant Exposure to Mustard Gas - Inhalation

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (mg/m3)	LOAEL Less serious (mg/m3)	LOAEL Serious (mg/m3)	Reference Chemical Form
ACUTE EXPOSURE							
Systemic							
1	Mouse (albino)	1 h	Renal		21.3 F increased blood and urine uric acid levels		Kumar and Vijayaraghavan 1998
2	Mouse (albino)	1 h	Resp		84.6 F decreased lung/Bd Wt ratio		Pant and Vijayaraghavan 1999
			Bd Wt		84.6 F 14% reduction		
3	Mouse (albino)	1 h	Resp		21.3[b] F decreased respiratory frequency		Vijayaraghavan 1997
			Bd Wt			16.9 F 28% reduction	
4	Gn Pig Not reported	10 min	Bd Wt		125 14% reduction		Allon et al. 1993
Immunological/Lymphoreticular							
5	Mouse (albino)	1 h			84.6 F decreased spleen/Bd Wt ratio		Pant and Vijayaraghavan 1999

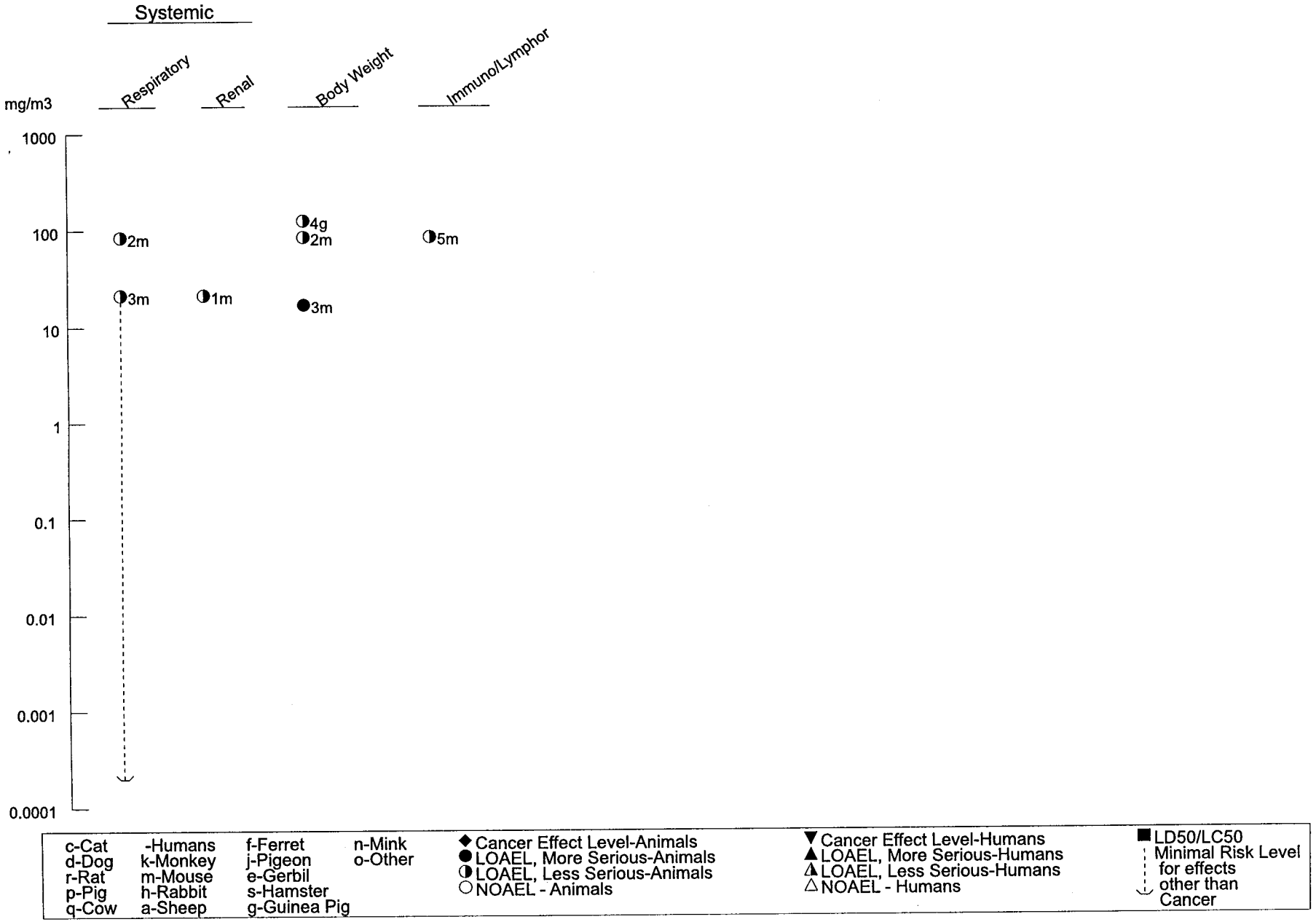
[a]The number corresponds to entries in Figure 3-1.

b Used to derive an acute inhalation MRL of 0.0002 mg/m^3; concentration duration adjusted to a 24-hour exposure period, dosimetrically adjusted for humans, and divided by an uncertainty factor of 300 [10 for use of a LOAEL, 3 for extrapolation from animals to humans using a dosimetric adjustment, and 10 for human variability] and a modifying factor of 3 [for proximity to serious effects (28% body weight loss at 16.9 mg/m^3)] (see Appendix A).

Bd Wt = body weight; F = female; Gn Pig = Guinea Pig; h = hour(s); LOAEL = lowest-observed-adverse-effect level; mg/m3 = milligram per cubic meter; NOAEL = no-observed-adverse-effect level; Resp = respiratory

Figure 3-1. Levels of Significant Exposure to Mustard Gas - Inhalation

Acute (≤14 days)



stenosis, bronchiectasis, bronchiolitis, and bronchitis. British soldiers exposed to mustard gas during combat in World War I had a significantly higher incidence of death due to bronchitis than the general population (Case and Lea 1955).

A group of patients, including a subgroup of 14 children and teenagers (9 boys, aged 9 months to 14 years; 5 girls, aged 13 months to 9 years), were examined 18–24 hours following exposure to mustard gas from air bombs during the Iran-Iraq War (Momeni and Aminjavaheri 1994). Cough, an early symptom and the most prevalent acute respiratory effect, developed in 11 children (79%). Other respiratory effects included crepitation (57%), dyspenea (57%), wheezing (36%), and sore throat (14%). Children had higher occurrences of respiratory effects than adults.

Other studies show that factory workers who were apparently exposed to mustard gas for a few years (exact quantity and duration not reported) developed acute and chronic respiratory effects. Factory workers in Britain who were exposed to mustard gas also showed increased deaths due to acute and chronic nonmalignant respiratory disease, including influenza and pneumonia (Easton et al. 1988). Workers in a Japanese poison gas factory were more likely to have chronic bronchitis, chronic cough, and decreased respiratory volume than nonexposed persons (Nishimoto et al. 1970). Manning et al. (1981) reported a significantly increased incidence of mortality from pneumonia among 428 former workers of a mustard gas manufacturing facility.

The development of chronic destructive pulmonary sequelae, 10 years after mustard gas vapor exposure, was reported in a clinical study of 197 veterans, admitted to the hospital in 1986 due to acute respiratory symptoms (Emad and Rezaian 1997). The patients' exposures to mustard gas were verified by studies of their urine and vesicular fluid. Patients were included in the study only if they had no history of smoking, cardiovascular disease, asthma, or exposure to other environmental agents known to cause interstitial lung disease or extrinsic allergic alveolitis. A control group was composed of 84 nonsmoking veterans who had participated in the Iran-Iraq War in another region in the same year (1986) but had no exposure to mustard gas. All study subjects had an ECG, a chest x-ray, a high-resolution CT of the chest, pulmonary function testing, and carbon monoxide diffusion capacity testing. Drug treatment was withdrawn for appropriate times prior to testing. Bronchial biopsies were done for each subject. Transbronchial lung biopsy was performed for 24/197 patients whose test data were suggestive of interstitial lung disease. The mean ages of the exposed and control groups were 34.39 and 35.52, respectively. Asthma was newly diagnosed in 21 (10.7%), chronic bronchitis in 116 (58.9%),

bronchiectasis in 17 (8.6%), airway narrowing due to scaring or granulation tissue in 19 (9.6%), and pulmonary fibrosis was observed in 24 (12.2%). None of these were found in the control group, with the exception of a single case of chronic bronchitis. A significant positive correlation was reported between the age of the subject and the severity of asthma, but not with the severity of pulmonary fibrosis. There was a significant correlation between age and incidence, but not the severity, of chronic bronchitis. There was a significant correlation between the severity of pulmonary fibrosis with the spirometry measurement of carbon monoxide diffusion capacity, but not the other physiological parameters of forced vital capacity (FVC) or forced expiratory volume in 1 second (FEV_1). No bronchial carcinoma or other lung malignancies were found in the veterans 10 years after exposure to mustard gas.

A retrospective mortality study was conducted in World War II veterans who participated in U.S. military experiments testing the effectiveness of various protective clothing and equipment in preventing injury due to mustard gas (Bullman and Kang 2000). The study identified 1,545 white male Navy recruits who were exposed to nonlethal levels (>120–960 mg-minute/L) of mustard gas at a single site between 1944 and 1945. A control group consisted of 2,663 white male Navy veterans who served at the same location and time as the exposed, but did not participate in mustard gas chamber tests. Mustard gas chamber test documentation included concentration of mustard gas in the chamber, length of exposure, and subject physiological reactions, so that a dose-response analysis could be conducted. The veterans who participated in the mustard gas chamber tests, while exposed to lower levels than estimated for combat exposed World War I veterans, did have sufficient exposure to produce skin reactions of erythema and edema. Causes of death investigated included laryngeal, lung, and skin cancers, chronic obstructive pulmonary and parenchymal respiratory diseases, external causes, and suicide. While reported as not statistically significant, the greatest mortality rate ratio, 1.57 (95% confidence interval [CI]=0.70–3.54) resulted for chronic obstructive pulmonary disease among veterans with exposure levels in the range of 121–960 mg-minute/L.

Short-term respiratory effects similar to those described in humans have been reported in experimental animals. Microscopic changes of the airways and lungs were reported in rabbits exposed to mustard gas vapor (Pechura and Rall 1993). Correlation of pathology with dose and time after exposure are not possible because of the wide variation in the concentration-time products employed and in the times of examination. However, the major pathological changes were in the nasal passages, pharynx, larynx, and upper portion of the respiratory tract, in some cases, the mouth, and in severe cases, the bronchi and bronchioles. These changes included superficial degeneration or necrosis of the epithelial lining with

pseudomembrane formation accompanied by congestion, edema, and increased mucus secretion. In animals with severe injuries that survived beyond a few days, the lesions incurred secondary infections, leading to bronchopneunomia, which apparently was the cause of death in many cases.

Dogs exposed to unspecified levels of mustard gas developed irregular respiration 8 hours after exposure (Winternitz and Finney 1920). Animals that died 1–3 days after exposure displayed destruction of the epithelial lining, the presence of pseudomembrane, and leukocytic infiltration in the trachea and bronchi. Evidence of bronchopneunomia was present in dogs that died 2–10 days after exposure.

These reports indicate similar respiratory effects of mustard gas in the three species (rabbits, dogs, and human), which suggests that knowledge obtained regarding respiratory effects in animal models can be usefully applied to humans.

Mice exposed to mustard gas vapor exhibited acute and delayed respiratory effects (Vijayaraghavan 1997). Groups of Swiss albino mice (four mice/group, 24–26 g) were administered mustard gas (>99% purity, dissolved in acetone and nebulized) 1 time by inhalation (head only) to 8.5, 16.9, 21.3, 26.8, 42.3, or 84.7 mg/m^3 for 1 hour. At all mustard gas concentrations administered, mice exhibited sensory irritation, 15–20 minutes after the start, characterized by a pause between inspiration and expiration. The respiratory frequency decreased to a slower steady state after 30 minutes of exposure, a decrease of approximately 20% at the lowest concentration to a maximum of 64% for concentrations $42.3 mg/m^3. The concentration that depressed 50% of the respiratory frequency (RD_{50}) was calculated as 27.4 mg/m^3. Normal respiration pattern was recovered after inhalation exposure was terminated; no pauses between respiratory cycles were measured at any exposure level, from which the authors concluded a lack of pulmonary irritation and toxic effects limited to the upper respiratory tract. While sensory irritation was reversible, delayed effects of mustard gas were indicated by a significant reduction in respiratory frequency beginning 48 hours after exposure at concentrations of 21.3 mg/m^3 and higher. The depression in respiratory frequency following exposure was related to both concentration and postexposure time. Airflow limitation was evidenced by a lengthening of expiration time and a decreased respiratory rate and is thought to occur due to the effect of mustard gas on the tracheal secretory cells. Reversible respiratory effects were also observed in similar experiments in mice by Rao et al. (1999) (10.6–42.3 mg/m^3) and by Pant and Vijayaraghavan (1999) (84.6 mg/m^3).

Guinea pigs were exposed by inhalation to 1,200–1,900 µg-minute/L of mustard gas for 10 minutes (120–190 mg/m^3) (Allon et al. 1993). A decrease in respiratory rate and minute volume, and an increase in tidal volume occurred immediately after the onset of exposure and lasted for up to 7 days after exposure. The changes in respiratory parameters were accompanied by a significant reduction in oxygen diffusion capacity in the lung. A dose-related decrease in body weight (14–27%) was also observed, with no recovery evident at 7 days postexposure. Pant and Vijayaraghavan (1999) measured a significant 13% reduction in lung-to-body weight ratio in mice exposed to 84.7 mg/m^3 for 1 hour.

Cardiovascular Effects. In 12 of 53 (23%) autopsies of victims of the World War II Bari Harbor incident during which mustard gas was released in to the air and water, small sub-epithelial hemorrhages were noted in the hearts, but in all instances, the parietal pericardium showed no pathology (Alexander 1947). There was a slight increase in the pericardial fluid having normal color in four cases (8%). In 18 cases, the myocardium was described as pale and lacking normal firmness.

Studies of 65 mustard gas casualties of the Iran-Iraq War treated in European hospitals did not indicate any heart abnormalities (Willems 1989). However, mild tachycardia without fever was reported in a group of 14 children and teenagers (9 boys, aged 9 months to 14 years; 5 girls, aged 13 months to 9 years) that were examined in a hospital in Iran 18–24 hours following exposure to mustard gas from air bombs during the Iran-Iraq War (incidence not reported) (Momeni and Aminjavaheri 1994). In a 1996 follow-up study of Iran-Iraq War veterans, 10 years after hospital admission in 1986 due to acute respiratory symptoms with confirmed mustard gas exposure, only 3/212 (1.4%) had cardiovascular disease, which was not confirmed attributable to exposure (Emad and Rezaian 1997) (see study description under Respiratory effects).

Gastrointestinal Effects. Victims of the World War II Bari Harbor incident, during which mustard gas was released in to the air and water, suffered local lesions of the oropharynx and upper portion of the esophagus (Alexander 1947). In a few cases, there was intense congestion of the first inch of the esophagus, which may or may not have been due to the blast. In 19 of 53 (36%) cases autopsied, stomach irritation and inflammation were documented. The lesions varied from simple hyperemia, to focal loss of epithelium, necrosis, and ulceration. Some lesions were located near the cardiac end, but most were in the region of the pylorus. In some cases, the hyperemia extended into the duodenum, and in one case, congestion of the jejunum was noted (Alexander 1947). Incidences of gastrointestinal effects of nausea (9 patients, 64%), vomiting (6 patients, 43%), and bleeding (2 patients, 14%) were reported in

a group of 14 children and teenagers (9 boys, aged 9 months to 14 years; 5 girls, aged 13 months to 9 years) that were admitted to a hospital in Iran 18–24 hours following exposure to mustard gas from air bombs during the Iran-Iraq War (Momeni and Aminjavaheri 1994). In a review of the clinical manifestations of mustard gas exposure in the Iran-Iraq War victims, Pierard et al. (1990) reported that endoscopy frequently revealed acute gastritis. Gastrointestinal neoplasms were reported in Japanese mustard gas factory workers who were involved with the production of chemical agents during World War II (Yamakido et al. 1985). Angelov et al. (1996) observed changes in the intestinal muscosa consisting of villi necrosis, dilatation of blood vessels, and increased cellular presence in broiler chickens after inhalation exposure to 0.9 mg/L (900 mg/m^3, 138 ppm) of mustard gas for 30 minutes.

Hematological Effects. There are reports of changes in white blood cell (WBC) counts in victims of mustard gas exposure during World War I and the Iran-Iraq War. During days 1–3 following exposure in World War I, increases of 3–5 times normal levels in WBC counts in peripheral blood were measured (Marrs et al. 1996). The increase was due mainly to an increase in polymorphonuclear cells, while lymphocytes were reduced in numbers during this period. In severe cases, a subsequent leukopenia occurred with WBC counts falling to <200 cells/µL. Leukopenia was also observed in casualties of mustard gas exposure of the World War II Bari harbor incident and during the Iran-Iraq War (Dacre and Goldman 1996; Marrs et al. 1996; Momeni and Aminjavaheri 1994). A 13-month-old Iranian girl developed pancytopenia and respiratory failure, and died 8 days after exposure (Momeni and Aminjavaheri 1994). In a group of 14 children and teenagers (9 boys, aged 9 months to 14 years; 5 girls, aged 13 months to 9 years) that were admitted to a hospital in Iran 18–24 hours following exposure to mustard gas from air bombs during the Iran-Iraq War, admission WBC counts ranged from 9,500 to 11,200 cells/µL, indicating mild leukocytosis (Momeni and Aminjavaheri 1994).

In a review of the clinical manifestations of mustard gas exposure in the Iran-Iraq War victims, Pierard et al. (1990) reported that in addition to the leukocytosis followed by leukopenia and lymphopenia described above, the ratio of T and B lymphocytes decreases, while the phagocytic function of neutrophils remains intact. A primary decrease in albumin and increase in α-globulin content, especially α_1 antitrypsin, occurs. Both C3 and C4 titers first increase, followed by a gradual decrease. Aplastic anemia is not uncommon. Increases in serum tumor markers, α-fetoprotein, β-HCG, and CA-125, have been observed, but the relevance of these increases to the oncogenic potential of mustard gas is not yet known.

The spleen demonstrated evidences of pathology in 33 of 53 (62%) autopsies of victims of the World War II Bari Harbor incident during which mustard gas was released in to the air and water (Alexander 1947). The majority were described as shrunken in size with pale color. Discoloration of the lymph nodes in the axillary, inguinal, and mesenteric glands were noted. The bone marrow was only examined in one autopsy and a pale pink color was described.

Cameron et al. (1946) provided a general description of pathological changes in rabbits and monkeys that had undergone tracheal cannulation and were exposed to nominal chamber concentrations of mustard gas ranging from 30 to 350 mg/m^3 (5–54 ppm). A large blood clot occupied most of the nasal passages in severe cases. In milder cases, damage was found only in the anterior turbinate bones and consisted of small hemorrhages in congested mucous membrane, with polymorphonuclear infiltration. The glands of Bowman and the organ of Jacobsen were consistently uninjured. Mustard gas vapors most often had little effect on the lungs; however, lethal effects occurred in the absence of lung damage.

Dogs and rabbits exposed to 0.1 mg/m^3 of mustard gas in the air for 1 year showed no hematological changes in a study that did not report further experimental details (McNamara et al. 1975).

Changes in the coloring and formation of erythrocyte nuclei and fatty dystrophy of bone marrow cells were observed in broiler chickens after inhalation exposure to 0.9 mg/L (900 mg/m^3, 138 ppm) of mustard gas for 30 minutes (Angelov et al. 1996).

Blood uric acid increased significantly in a dose- and time-related manner in female mice exposed by inhalation to 21.2, 42.3, or 84.6 mg/m^3 of mustard gas for 1 hour (Kumar and Vijayaraghavan 1998). Blood uric acid levels peaked at 2 days after exposure, but were still significantly elevated above controls at 7 days postexposure.

Musculoskeletal Effects. No evidence of mustard gas-related changes to the musculoskeletal system was reported in any of 53 autopsies of victims of the World War II Bari Harbor incident during which mustard gas was released in to the air and water (Alexander 1947).

Hepatic Effects. In 39 of 53 (74%) autopsies of victims of the World War II Bari Harbor incident, during which mustard gas was released in to the air and water, yellow streaks and patches grossly appearing as patchy fatty necrosis were observed throughout the liver (Alexander 1947). Several pale

liver sections and atypical liver texture were mentioned. In 3 of 53 (6%) autopsies, small subcapular hemorrhages, and in one instance, a small rupture near the diaphragmatic attachment, were noted. The gall bladder contained thick inspissated bile. Microscopic examinations were performed on 31 of the 39 livers with gross changes. Five showed fatty change and two showed focal necroses.

Renal Effects. Renal complications, consisting of acute hemorrhagic nephritis, oliguria, albuminuria, and casts, have been reported in near-death stages of mustard gas warfare victims (Papirmeister et al. 1991).

In 25 of 32 (78%) kidneys from Bari Harbor incident casualties, microscopic examinations revealed tubular casts, 3 were calcified, 8 showed hemoglobin casts, and in 14, both types were present (Alexander 1947).

Blood uric acid increased significantly in a dose- and time-related manner mice, indicative of kidney damage, in females exposed by inhalation to 21.2, 42.3, or 84.6 mg/m^3 of mustard gas for 1 hour (Kumar and Vijayaraghavan 1998). Blood uric acid levels peaked at 2 days after exposure, but were still significantly elevated above controls at 7 days postexposure.

Endocrine Effects. No significant findings were noted grossly in the thyroid or adrenal glands in any of 53 autopsies of victims of the World War II Bari Harbor incident (Alexander 1947).

The time course of changes in serum concentrations of total and free testosterone, lutenizing hormone (LH), dehydroepiandrosterone (DS), follicle-stimulating hormone (FSH), 17 α-OH progesterone, and prolactin were studied in 16 men during the first 3 months after chemically confirmed exposure in 1987 during the Iran-Iraq War to chemical weapons containing mustard gas (Azizi et al. 1995). A group of 34 healthy unexposed men of similar age served as controls. At 1 week after exposure, total testosterone, free testosterone, and DS were significantly lower, 57, 72, and 53%, respectively, in exposed men than in controls, while levels of the remaining hormones were comparable between groups. Total testosterone, free testosterone, and DS levels continued to decrease during the first 5 weeks after exposure. At 1 week, 4 of 16 exposed men (25%) had serum testosterone levels that were reduced by >60% below the control average; by the 5^{th} week, the number increased to 11 (69%). DS mean values reached as low as 18% of the mean of control subjects. After the 5^{th} week, these three hormone levels increased returning to normal levels at 12 weeks after injury. Small but significant increases in mean

serum concentration of LH at the 3rd week and that of FSH and prolactin at the 5th week, were measured. Normal levels of LH, FSH, and prolactin were measured at 12 weeks. FSH and LH response levels to 100 µg of gonadotropin releasing hormone (GnRH) administered intravenously during the first week after exposure, were subnormal in four of five patients.

In another study, the time course of changes in thyroid indices, serum T3, T4, TSH, reverse T3, thyroglobulin and cortisol, plasma adrenocorticotropic hormone (ACTH), and free T3 and T4 indexes (FT3I, FT4I) were studied in 13 male soldiers, ages 21–32 years, during the first 5 weeks after chemically confirmed exposure in 1987 during the Iran-Iraq War to chemical weapons containing mustard gas (Azizi et al. 1993). A group of 34 healthy unexposed men of similar age served as controls. T4 and FT4I were not consistently affected following injury; compared to controls, significantly decreased values were measured at 1 and 5 weeks after exposure, but values slightly above normal were measured at 3 weeks. T3 and FT3I were significantly lower (11–23%) than control at 1, 3, and 5 weeks after injury. Reverse T3 concentration in injured men was significantly higher (29%) than mean control value at 1 week, but was normal at weeks 3 and 5. TSH and thyroglobulin levels in the injured soldiers were comparable to controls during the 5 postexposure weeks. Cortisol was significantly higher (40%) than normal 1 week after exposure, within the normal range at week 3, and significantly decreased (50%) below normal at week 5. ACTH was significantly increased (57–80%) above the normal control value at 1, 3, and 5 weeks after exposure.

In a follow-up study of 42 men, ages 18–37, injured by mustard gas during the Iran-Iraq War, serum testosterone, LH, and prolactin concentrations were normal in all men 1–3 years following exposure (Azizi et al. 1995). A comparison of the mean serum FSH concentration in 13 subjects with sperm count below 20 million and in 20 subjects with sperm counts above 60 million, revealed a nearly 2-fold increase in FSH concentration in the those with the lower sperm count; the increased FSH level was 38% above the mean FSH concentration in a group of 34 health unexposed males.

Dermal Effects. The U.S. Army Soldier and Biological Chemical Command (SBCCOM 1999) reports a maximum safe concentration time product (Ct) of 5 mg-minute/m^3 for human skin exposure to mustard gas. The ICt_{50} (estimated concentration-exposure time period product incapacitating to 50% of exposed individuals) for human skin exposure is dependent on temperature, 2,000 mg-minute/m^3 at 70–80 EF (humid environment) and 1,000 mg-minute/m^3 at 90 EF (dry environment) (SBCCOM 1999).

Ocular Effects. In humans, an ICt_{50} and a maximum safe Ct for eye exposure are 200 and 2 mg-minute/m^3, respectively, for mustard gas (SBCCOM 1999).

Body Weight Effects. In female Swiss albino mice exposed to mustard gas for by inhalation (head only) at concentrations of 8.5, 16.9, 21.3, 26.8, 42.3, or 84.7 mg/m^3 for 1 hour, decreases in body weight began 24 hours after exposure, were concentration-related, and achieved statistical significance ($p<0.05$) at concentrations of 16.9 mg/m^3 or higher (Vijayaraghavan 1997). At 7 days postexposure, body weights were decreased by 2, 13, 28, 25, 32, and 34% in the control, 8.5, 16.9, 21.3, 26.8, and 42.3 mg/m^3 exposure groups. In another study in female albino mice, in which mustard gas was administered at 84.6 mg/m3 for 1 h., a progressive fall in body weight was observed starting on the third post-exposure day, and at post-exposure day 7, body weight was significantly reduced by 14%, compared to control animals (Pant and Vijayaraghavan 1999). Food and water intake was also significantly decreased.

Guinea pigs administered nominal concentrations of 1250, 1650, or 1750 µg-min/l (125, 165, or 175 mg/m^3) of mustard gas (head only) for 10 minutes exhibited a dose-related significant decrease in body weight, with no recovery evident at 6-7 days post-exposure (Allon et al. 1993). At 6-7 days post-exposure, body weight was reduced compared to controls by -14%, -24%, and -27% at the low-, mid-, and high-concentrations, respectively (data presented graphically).

3.2.1.3 Immunological and Lymphoreticular Effects

The spleen demonstrated evidences of gross pathology in 33 of 53 (62%) autopsies of victims of the World War II Bari Harbor incident during which mustard gas was released in to the air and water (Alexander 1947). In the majority of cases, the spleen was described as shrunken in size with pale color. Discoloration of the lymph nodes in the axillary, inguinal, and mesenteric glands were noted. No significant findings were noted grossly in the thymus in any of the autopsies. Microscopically only 2 of 32 spleens examined showed degeneration or necrosis; pyknosis and karyorrhexis of lymphocytes in some corpuscles was observed in one and slight necrosis of the malpighian follicle in the other.

Cameron et al. (1946) provided a general description of pathological changes in rabbits and monkeys that had undergone tracheal cannulation and were exposed to nominal chamber concentrations of mustard gas ranging from 30 to 350 mg/m^3 (5–54 ppm). After 12 hours, damage was found in the cervical lymph nodes, which drain the nose and lymphoid tissue throughout the body. In experiments where the time

sequence was studied, damage to the cervical lymph nodes could not be attributed solely to lymphatic absorption from nasal mucosa, since identical changes resulted from topical skin application or subcutaneous injection of mustard gas.

Angelov et al. (1996) detected atrophy of the lymphoid tissue in the bursa Fabricii of broiler chickens after inhalation exposure to 0.9 mg/L (900 mg/m^3, 138 ppm) of mustard gas for 30 minutes.

Pant and Vijayaraghavan (1999) measured a significant 38% reduction in spleen-to-body weight ratio in mice exposed to 84.7 mg/m^3 for 1 hour.

3.2.1.4 Neurological Effects

Nausea and vomiting are acute responses to mustard gas exposure in adults and children. Nausea and vomiting occurred, respectively, in 9 (64%) and 6 (43%) of 14 Iranian children admitted to a hospital following exposure to mustard gas from air bombs during the Iran-Iraq War (Momeni and Aminjavaheri 1994).

No significant findings were noted grossly in the central nervous system in any of 53 autopsies of victims of the World War II Bari Harbor (Alexander 1947).

Dogs exposed to unspecified levels of mustard gas showed no tremors or convulsions (Winternitz and Finney 1920).

3.2.1.5 Reproductive Effects

In a follow-up study of 42 men, ages 18–37, conducted 1–3 years after injury by mustard gas during the Iran-Iraq War, the mean sperm count was 84 million cells per mL, ranging from 0 to 328 million cells per mL (Azizi et al. 1995). Thirteen (29%) had decreased sperm count below 20 million. Serum testosterone, LH, and prolactin concentrations in the 13 subjects with sperm count below 20 million were comparable to the levels in 20 subjects with sperm count above 60 million. FSH measured in these same groups was higher in the group with lower sperm counts. The increased FSH level was 38% above the mean FSH concentration in a group of 34 healthy unexposed males. Complete or relative arrest of

spermatogenesis was evident in each testicular biopsy (100% incidence) performed on six men with sperm count below 20 million cells per mL.

Pour-Jafari (1992, 1994a) reported an increased rate of fetal deaths and an increased secondary sex ratio (57.2 vs. 51.0% in controls, percent of males) in progenies of Iranian survivors of chemical attacks that included mustard gas.

In a survey of 800 Iranian men who were exposed to mustard gas during the Iran-Iraq War, 279 men (34.8%) reported decreased libido, 342 (42.8%) reported no change, 6 (0.8%) reported increased libido, and 173 (21.6%) did not respond to this survey question (Pour-Jafari and Moushtaghi 1992). Of these men, 86.6% still suffered symptoms from chemical injury, namely lung and skin lesions.

Chronic (52 weeks) inhalation exposure of male rats to mustard gas (0.1 mg/m^3) was reported to produce significant dominant lethal mutation rates (a maximum of 9.4% at 12–52 weeks), but exposure of pregnant females to the same concentration for a shorter time interval did not (Rozmiarek et al. 1973). McNamara et al. (1975) subsequently concluded from these same data that there were no differences between the control and experimental groups and no evidence of mutagenesis. The conflict between these two reports is not readily resolvable, but the fetal mortality values presented by McNamara et al. (1975) suggest at least a trend for dominant lethal effect. Complete control data and statistical analyses of the results were not presented, but percentages of fetal death at week 12 were 4.12, 4.24, and 21.05 for controls, 0.001, and 0.1 mg/m^3 exposure groups, respectively.

3.2.1.6 Developmental Effects

Pour-Jafari (1994b) reported an increased incidence of congenital malformations among offspring of Iranian mustard gas victims.

No excess in fetal abnormalities were noted when rat dams were exposed to mustard gas by inhalation during gestation (McNamara et al. 1975). This study had a number of drawbacks, including failure to report humidity in the chamber (which made an adequate assessment of exposure levels difficult), whole-body exposure of animals (which made the exposure a combination of inhalation as well as ingestion effects), additions of animals in the chamber midway through the experiment (which allowed for entrance of pathological organisms), lack of rationale for the selection of dosages, lack of details of

pathological examination (which normally includes description of the types of defects the investigators were looking for), lack of information about historical controls, and lack of statistical evaluations. In particular, the authors stated that the fetuses were examined, but they did not indicate whether or not there were any fetal abnormalities.

3.2.1.7 Cancer

Human Cancer Studies. Data on cancer in humans after inhalation exposure to mustard gas are from two primary sources: inhalation for several years by mustard gas factory workers and inhalation as the result of a few or of single exposures during combat in World War I and in the Iran-Iraq War. While several epidemiologic studies provide sufficient evidence that mustard gas is carcinogenic in humans, particularly in the upper respiratory tract, in no case was the exposure level or duration quantified, and therefore, these data are inadequate for deriving dose-response relationships. Typically, factories produced several different poisonous gases and workers involved with mustard gas production were exposed to other toxic chemicals, confounding any study findings.

Other studies provide epidemiological evidence that World War I veterans who were exposed to mustard gas in combat had slight, but statistically significant, increased incidences of lung cancer deaths. British retired veterans who were studied 15 years after their exposure to mustard gas in World War I showed twice the expected number of deaths due to lung cancer (standard mortality ratio [SMR]=2; $p<0.01$) compared to controls and also had excessive deaths from bronchitis (SMR=10, $p<0.001$), as compared to nonexposed soldiers (Case and Lea 1955). The authors suggest that the increased lung cancer was due to the bronchitis and not directly to the mustard gas. Veterans who were not exposed to mustard gas, but who did have bronchitis also had excess mortality due to lung cancer (SMR=2; $p<0.01$), as compared with controls. Deaths from neoplasms other than cancer of the lung were not significantly increased.

A cohort of American World War I soldiers was studied 1–37 years (Beebe 1960) and 47 years (Norman 1975) postexposure. Deaths from respiratory cancer occurred in 2.5% of those exposed to mustard gas, 1.8% of those having pneumonia, and in 1.9% of a control group (Norman 1975). The respiratory cancer rate ratio of 1.3 (95% CI=0.9–1.9) is suggestive evidence of an association of lung cancer with mustard gas exposure. These studies found no association of lung cancer with bronchitis, in contrast to the findings of Case and Lea (1955). Although an increased frequency of lung cancer is associated with

exposure to mustard gas, it is very difficult to calculate attributable risk of lung cancer due to mustard gas, as no control was made for cigarette smoking.

A retrospective mortality study was conducted in World War II veterans who participated in U.S. military experiments testing the effectiveness of various protective clothing and equipment in preventing injury due to mustard gas (Bullman and Kang 2000). The study identified 1,545 white male Navy recruits who were exposed to nonlethal levels (>120–960 mg-minute/L) of mustard gas at a single site between 1944 and 1945. A control group consisted of 2,663 white male Navy veterans who served at the same location and time as the exposed, but did not participate in mustard gas chamber tests. Mustard gas chamber test documentation included concentration of mustard gas in the chamber, length of exposure, and subject physiological reactions, so that a dose-response analysis could be conducted. The veterans who participated in the mustard gas chamber tests, while exposed to lower levels than estimated for combat exposed World War I veterans, did have sufficient exposure to produce skin reactions of erythema and edema. Causes of death investigated included laryngeal, lung, and skin cancers, chronic obstructive pulmonary and parenchymal respiratory diseases, external causes, and suicide. The mortality rate ratios for all cancer types among the total exposure group and all subgroups were less than unity. The greatest mortality rate ratio, 1.57 (95% CI=0.70–3.54) resulted for chronic obstructive pulmonary disease among veterans with exposure levels in the range of 121–960 mg-minute/L. The authors indicated that this value was not statistically significant and that there was no excess of any cause-specific mortality associated with mustard gas exposure or associated with level of mustard gas exposure among veterans. The authors noted that reliance on death certificates for cause of death and lack of data on potential confounders (smoking, drinking habits, and occupational history/exposure to carcinogens) were potential study weaknesses.

In a 1996 follow-up clinical study of 197 Iran-Iraq War veterans, 10 years after hospital admission in 1986 due to acute respiratory symptoms with confirmed mustard gas exposure, no bronchial carcinoma or other lung malignancies were found (Emad and Rezaian 1997) (see study description under respiratory effects in Section 3.2.1.2).

Studies from three countries show elevated incidence of lung cancer among factory workers who made mustard gas and other chemical agents. In Japanese factory workers, histological examination revealed foci of moderate or severe atypical cell lesions or carcinoma in the bronchial epithelium (Tokuoka et al. 1986). Another study of workers from this same factory showed an increased number of deaths

(SMR=37; 33 deaths observed vs. 0.9 deaths expected) from cancer of the respiratory passages (Wada et al. 1968). The neoplasms were of either the squamous or undifferentiated type. In another study of Japanese factory workers, with estimated factory mustard gas concentrations of 0.05–0.07 mg/L (0.0017–0.0024 mg/kg/day, for an average 70 kg body weight, 8-hour day, and 5-day work week) (Nakamura 1956), of 172 worker deaths, 48 (28%) were due to malignant tumors compared with 7.7 and 8.5% in two groups of unexposed residents of the same area (Yamada 1963). Respiratory tract tumors accounted for 58% of all malignant tumors (16% of all deaths). In the two reference groups, the incidence of respiratory tumors was much lower, 0.5 and 0.3%, respectively. In Japanese mustard gas cases, central lung cancers were more commonly observed than peripheral lung cancers, and the most common histologic types were squamous cell carcinoma and small cell carcinoma (Yamada 1963). The duration of mustard gas exposure in cases of lung cancer was 7–9 years, and the latent period for tumor induction was 17–20 years.

Two historical cohort studies were conducted to determine the comparative risk for development of cancer in Japanese males who worked in a poison gas factory between 1927 and 1945 (Nishimoto et al. 1983; Yamakido et al. 1996). The gases produced at the factory included mustard gas, lewisite, diphenylcyanarsine, hydrocyanic acid, chloracetophenone, and phosgene. No estimates of chemical exposures levels were given. However, the workers were divided into three groups according to type of work and association with mustard gas in an attempt to establish a dose-relationship. One group consisted of workers engaged directly in the production of mustard gas and lewisite. A second group consisted of workers who had come into contact with mustard gas and/or lewisite in laboratories or during repair or inspection in the factory. A third group consisted of those engaged directly in the production of the remaining gases, other than mustard gas and lewisite, or who were working in medical or administrative work. Nishimoto et al. (1983) investigated 2,068 cases and found that the number of deaths from cancer of the lungs in the two groups with the highest mustard gas exposure potential was more than 3 times the number in the local male population (SMR$3, $p<0.01$). Deaths due to cancers of the gastrointestinal tract and liver or other type were not significantly elevated. Yamakido et al. (1996) studied 1,632 male workers from this same factory. In this study, in addition to grouping according to type of work, groups were further subdivided according to the duration of work in the factory, <0.5 years, 0.5–5 years, or >5 years. The SMRs for lung cancer were significant ($p<0.001$) in the group working directly in the production of mustard gas and lewisite for >6 months (SMR=3.24 [0.5–5 years], SMR=7.35 [>5 years]). In the second grouping of workers who had less contact with mustard gas, the SMR for lung cancer was significant only in the subgroup with >5 years of employment (SMR=4.92),

further supporting a dose-relationship for lung cancer. However, there were no data presented to relatively weight the exposure to mustard gas and lewisite within the work type grouping; therefore, co-exposure to lewisite confounds the interpretation of these findings. The SMR for all malignant neoplasms was significant in the group with the highest mustard gas exposure potential with >6 months of employment (SMR=1.44 [0.5–5 year, $p<0.05$], SMR=2.36 [>5 year, $p<0.001$]) and in the second grouping of workers who had less contact with mustard gas but worked at the factory for the longest duration (SMR=1.66 [>5 year, $p<0.05$]).

British mustard gas workers also showed increased deaths from cancers of the respiratory passages and from lung cancer (Manning et al. 1981). In a cohort study of 502 workers involved in mustard gas manufacturing between 1940 and 1945, a significant excess mortality was observed for carcinoma of the larynx and trachea (SMR=7.5, $p<0.02$). While not listed as cause of death, seven subjects developed cancer of the larynx, compared with 0.75 expected, yielding a rate ratio of 9.3 ($p<0.001$). Increased mortality due to cancers of other organs was not statistically significant. In another study of 3,354 British mustard gas workers, significant excesses were observed compared with national death rates for deaths from cancer of the larynx (SMR=2.7, 11 deaths observed, 4.04 deaths expected, $p=0.003$), pharynx (SMR=5.5, 15 observed, 2.73 expected, $p<0.001$), lung (SMR=1.4, 200 observed, 138.39 expected, $p<0.001$), upper respiratory sites combined (lip, tongue, salivary gland, mouth, and nose) (SMR=2.8, 12 observed, 4.29 expected, $p=0.002$), esophagus (SMR=1.9, 20 observed, 10.72 expected, $p<0.01$), and stomach (SMR=1.4, 70 observed, 49.57 expected, $p<0.001$) (Easton et al. 1988). The risks of cancers of the pharynx and lung, but not of the esophagus and stomach, were significantly related to duration of employment.

A study of workers in U.S. mustard gas factories reported incidences of chronic bronchitis after 3–6 months of employment, but did not mention any incidence of lung cancer (Morgenstern et al. 1947). German factory workers also showed increases in bronchial carcinoma, bladder carcinoma, and leukemia (Weiss and Weiss 1975).

Animal Cancer Studies. Two animal studies showed tumors following inhalation exposure to mustard gas. Male and female Strain A mice exposed once for 15 minutes to an unquantified level of mustard gas had a significantly higher incidence of pulmonary tumors than did their littermate controls (Heston 1953b). The significance of this finding for humans is difficult to determine since these Strain A mice are used due to their specific genetic tendency to develop lung tumors. Guinea pigs, mice, rabbits, and

dogs that were exposed to mustard gas in the air for 3–12 months did not develop tumors, although rats did develop squamous cell carcinoma of the skin (McNamara et al. 1975). This study used insufficient animals and inadequate doses to be considered an adequate carcinogenesis assay.

3.2.2 Oral Exposure

No studies were located regarding the health effects in humans after oral exposure to mustard gas. While exposure to mustard gas by the oral route can occur, dermal or inhalation exposure is more prevalent.

3.2.2.1 Death

In humans, the LD_{50} for oral exposure is estimated to be 0.7 mg/kg (SBCCOM 1999).

Significant maternal mortality occurred in a teratology study in which mustard gas was administered acutely by oral gavage to mated female animals of two species, rats and rabbits, on gestation days 6 through 15 and 6 through 19, respectively (DOA 1987b). Rabbits were dosed with 0, 0.5, 1.0, 2.0, or 2.5 mg/kg/day of mustard gas in a range-finding study and with 0, 0.4, 0.6, or 0.8 mg/kg/day in the teratology study. In rabbits, maternal mortality was dose-related with mustard gas-related deaths occurring with a dose of 0.8 mg/kg/day or higher, 3/18 (17%) at 0.8 mg/kg/day, 3/7 (43%) at 1.0 mg/kg/day, 5/8 (63%) at 2.0 mg/kg/day, and 4/6 (75%) at 2.5 mg/kg/day. In the range-finding study, female rats were dosed with 0, 0.2, 0.4, 0.8, 1.6, 2.0, or 2.5 mg/kg/day and with 0, 0.5, 1.0, or 2.0 mg/kg/day in the teratology study. One of three rats died on gestation day 12 at the highest dose of 2.5 mg/kg/day. No maternal deaths in rats were attributed to mustard gas at doses below 2.5 mg/kg/day.

No mustard gas-related mortality occurred at doses of 0.5 mg/kg/day or less in three intermediate-duration toxicity studies in rats (Sasser et al. 1993, 1996a, 1996b). In a sub-chronic toxicity study, groups of 6–7-week-old Sprague-Dawley rats (12/sex/group) were orally gavaged with 0, 0.003, 0.01, 0.03, 0.1, or 0.3 mg/kg/day mustard gas in sesame oil, 5 days/week for 13 weeks (Sasser et al. 1996b). In a two-generation study, groups of 8-week old Sprague-Dawley rats (27 female and 20 males/ group/generation) were orally gavaged with 0, 0.03, 0.1, or 0.4 mg/kg/day mustard gas in sesame oil (Sasser et al. 1996a). Male and female rats were dosed 5 days/week for 13 weeks before mating and during a 2-week mating period. Females were dosed daily (7 days/week) throughout the 21-day gestation and parturition period and 4–5 days/week during the 21-day lactation period. Male and female F1 pups

were treated with mustard gas until they were mated and the females became pregnant and gave birth. The dosing of F1 dams continued until pup weaning, at which time, the study was terminated. In a dominant lethal study, groups of 7–10-week-old Sprague-Dawley rats were orally gavaged with 0.08, 0.2, or 0.5 mg/kg/day mustard gas in sesame oil for 5 days/week for 10 weeks (Sasser et al. 1993). In the female dominant lethal phase, groups of rats (40 females and 10 males/group) were administered 0 (sesame oil), 0.08, 0.2, or 0.5 mg/kg/day mustard gas for 5 days/week for 10 weeks prior to a 19-day breeding interval. In the male dominant lethal phase, groups of rats were administered 0 (sesame oil), 0.08, 0.2, or 0.5 mg/kg/day mustard gas for 5 days/week for 10 weeks.

The highest NOAEL and all LOAEL values for each study for death in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.2 Systemic Effects

The highest NOAEL and all LOAEL values for each study for systemic effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2. No studies were located regarding musculoskeletal, hepatic, renal, or metabolic effects in humans or animals after oral exposure to mustard gas.

Respiratory Effects. Gross examinations of the lungs of rats orally gavaged with 0.3 mg/kg/day mustard gas 5 days/week for 13 weeks did not reveal any significant treatment related lesions (Sasser et al. 1996b).

Cardiovascular Effects. Microscopic examinations of the heart of rats orally gavaged with 0.3 mg/kg/day mustard gas 5 days/week for 13 weeks did not reveal any significant treatment related lesions (Sasser et al. 1996b).

Gastrointestinal Effects. Dose-related gastrointestinal effects have occurred in experimental animals following acute and subchronic oral administration of mustard gas. In mated female rats orally gavaged with 0.2–2.5 mg/kg/day of mustard gas on gestation days 6 through 15, gastric mucosa inflammation was observed in 2/30 (6.7%) and 2/3 (66.7%) rats at 2.0 and 2.5 mg/kg/day, respectively, but not in any of the lower dose or control groups (DOA 1987b). Inseminated female rabbits orally gavaged with 0.4–2.5 mg/kg/day of mustard gas on gestation days 6 through 19 incurred dose-related

Table 3-2. Levels of Significant Exposure to Mustard Gas - Oral

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	ACUTE EXPOSURE						
	Systemic						
1	Rat (Sprague-Dawley)	10 d Gd 6-15 (GO)	Hemato	0.5 F		1.0 F (decrease in hematocrit)	DOA 1987b
			Dermal	2.0 F			
2	Rabbit (NS)	14 d Gd 6-19 (GO)	Hemato	0.6 F		0.8 (decrease in hematocrit)	DOA 1987b
			Dermal	0.8 F			
			Bd Wt	0.6 F	0.8 (7.9-10.5% decrease after 5 days of exposure)		
	Immunological/Lymphoreticular						
3	Rat (Sprague-Dawley)	10 d, Gd 6-15 (GO)			0.5[b] F (inflamed mesenteris lymph nodes)		DOA 1987b
	Developmental						
4	Rat (Sprague-Dawley)	10 d Gd 6-15 (GO)			0.5[b] (reduced ossification)		DOA 1987b
5	Rabbit (NS)	14 d Gd 6-19 (GO)		0.8			DOA 1987b

Table 3-2. Levels of Significant Exposure to Mustard Gas - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
INTERMEDIATE EXPOSURE							
Systemic							
6	Rat (Sprague-Dawley)	10 wk 5 d/wk 1 x/d	Dermal	0.5			Sasser et al. 1993
		(GO)	Bd Wt	0.2	0.5 M		
7	Rat (Sprague-Dawley)	18-21 wk 5 d/wk	Gastro		0.03 (29/47 M, 42/47 F; epithelial acanthosis of the forestomach)		Sasser et al. 1996a
		(GO)	Dermal	0.4			
8	Rat (Sprague-Dawley)	13 wk 5 d/wk 1 x/d	Resp	0.3			Sasser et al. 1996b
		(GO)	Cardio	0.3			
			Gastro	0.03	0.3[c] (5/12 M, 5/12 F; epithelial hyperplasia of the forestomach)		
			Hemato	0.1	0.3 F (8 % decrease in serum protein concentration)		
			Hepatic	0.3			
			Renal	0.3			
			Endocr	0.3			
			Dermal	0.3			
			Bd Wt	0.1	0.3 (>10% decrease in females, >8% decrease in males)		

Table 3-2. Levels of Significant Exposure to Mustard Gas - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL				Reference Chemical Form
					Less Serious (mg/kg/day)		Serious (mg/kg/day)		
	Immunological/Lymphoreticular								
9	Rat (Sprague-Dawley)	13 wk 5 d/wk 1 x/d (GO)		0.3					Sasser et al. 1996b
	Neurological								
10	Rat (Sprague-Dawley)	10 wk 5 d/wk 1 x/d (GO)		0.2	0.5	(excessive drooling)			Sasser et al. 1993
	Reproductive								
11	Rat (Sprague-Dawley)	10 wk 5 d/wk 1 x/d (GO)					0.5	(2-fold increase in abnormal sperm head morphology)	Sasser et al. 1993
							0.08	(4-fold increase in resorptions; increased preimplantation losses; 7% decrease in live fetuses)	
12	Rat (Sprague-Dawley)	18-21 wk 5 d/wk (GO)		0.1	0.4	(increased fraction of males, 58%)			Sasser et al. 1996a

Table 3-2. Levels of Significant Exposure to Mustard Gas - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
13	Rat (Sprague-Dawley)	13 wk 5 d/wk 1 x/d (GO)		0.3			Sasser et al. 1996b
	Developmental						
14	Rat (Sprague-Dawley)	18-21 wk 5 d/wk (GO)		0.4			Sasser et al. 1996a

[a]The number corresponds to entries in Figure 3-2.
[b]Used to derive an acute oral minimum risk level (MRL) of 0.0005 mg/kg/day; dose divided by an uncertainty factor of 1000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).
[c]Used to derive an intermediate-duration oral minimal risk level (MRL) of 0.00002 mg/kg-day; by adjusting for intermittent exposure (see Apendix A) and dividing by an uncertainty factor of 1000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).

Bd Wt = body weight; Cardio = cardiovascular; d = day(s); Endocr = endocrine; F = female; gastro = gastrointestinal; Gd = gestation day; (GO) = gavage in oil; Hemato = hematological; LOAEL = lowest-observed-adverse-effect level; M = male; mg/kg/day = milligram per kilogram per day; NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s); x = times

Figure 3-2. Levels of Significant Exposure to Mustard Gas - Oral

Acute (≤14 days)

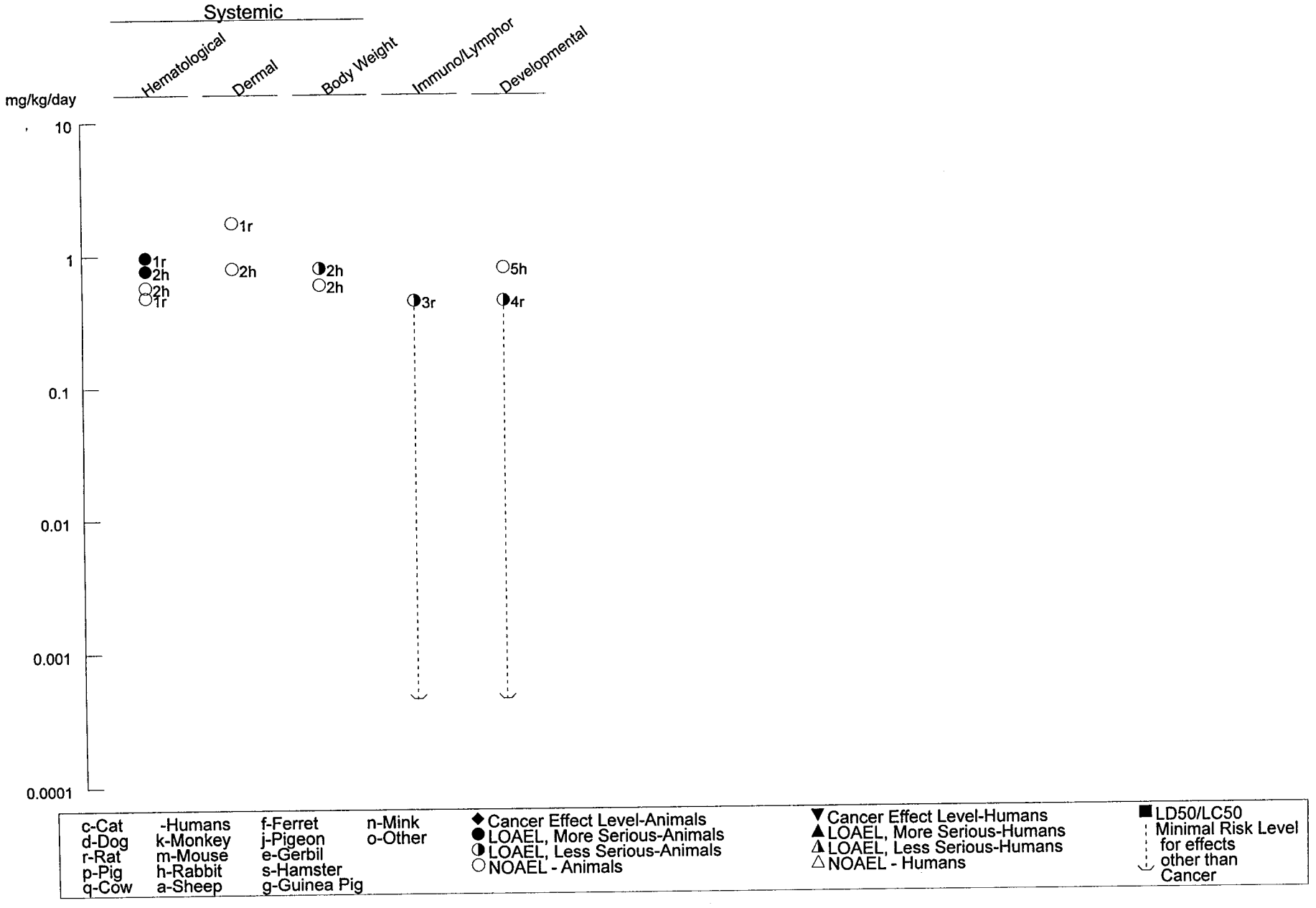
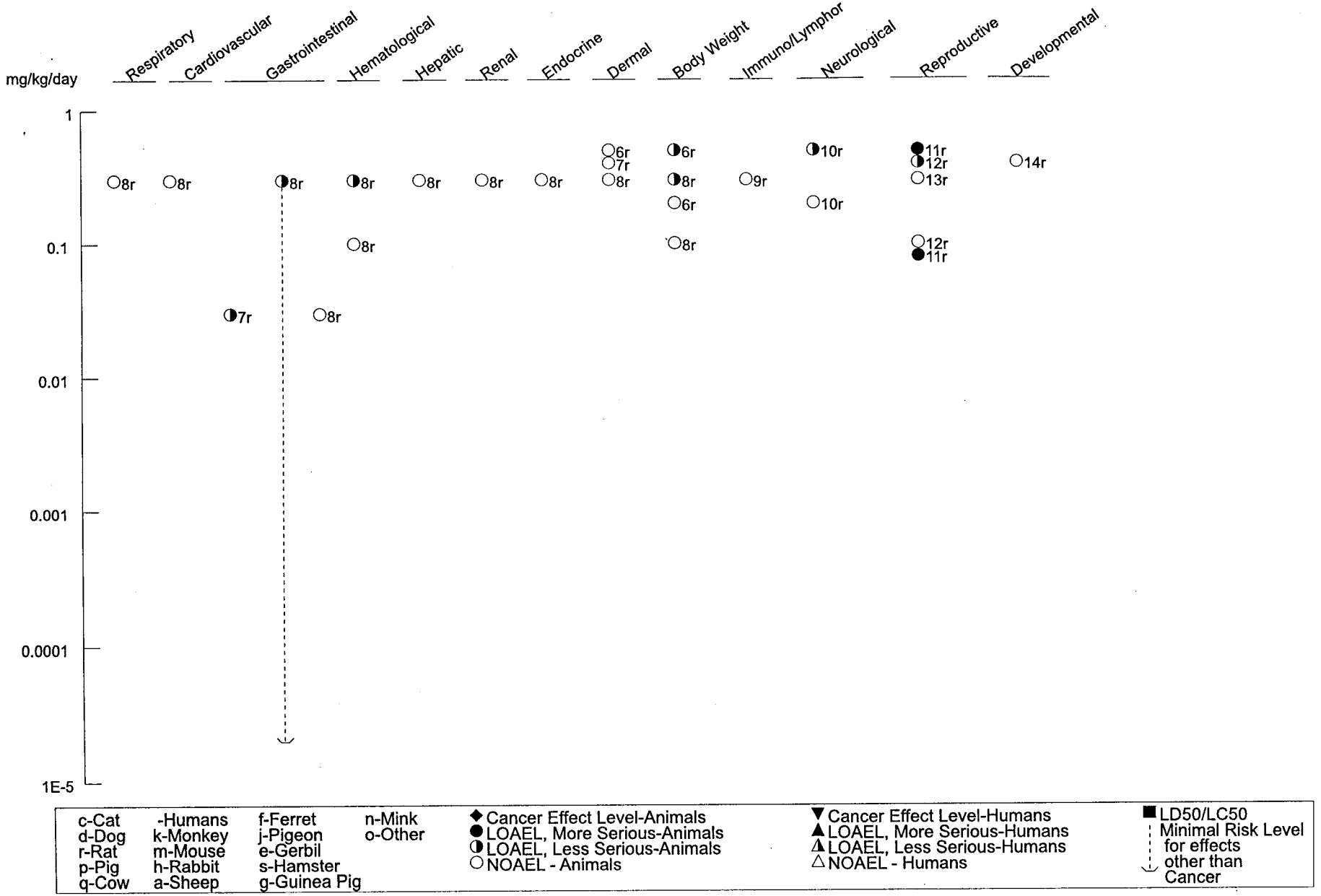


Figure 3-2. Levels of Significant Exposure to Mustard Gas - Oral (*continued*)

Intermediate (15-364 days)



damage to the gastric mucosa at doses of 0.4 mg/kg/day and higher. Gastric ulcers were observed in 0/19 controls and in 1/18 (5.6%), 0/8, 0/18, 3/18 (16.7%), 0/8, 2/8 (25.0%), and 1/7 (14.3%) rabbits at 0.4, 0.5, 0.6, 0.8, 1.0, 2.0, and 2.5 mg/kg/day, respectively (DOA 1987b).

Rats were orally gavaged with 0.003, 0.01, 0.03, 0.1, or 0.3 mg/kg/day mustard gas for 5 days/week for 13 weeks (Sasser et al. 1996b). Microscopic examinations revealed epithelial hyperplasia of the forestomach in 10/24 (41.7%) animals (5/sex) in the highest-dose group and in one male (1/24, 4.2%) at 0.1 mg/kg. Lesions were not found in any females in the 0.1 mg/kg group or in either sex of the 0.03 mg/kg group; therefore, the forestomaches of the lower dose groups were not examined. The hyperplastic change was characterized by cellular disorganization of the basilar layer, apparent increase in mitotic activity of the basilar epithelial cells, and thickening of the epithelial layer.

Dose-related incidence and severity of lesions of the squamous epithelium of the forestomach occurred in both sexes of rats orally gavaged with 0.03, 0.1, or 0.4 mg/kg/day mustard gas for 18–21 weeks (Sasser et al. 1996a). The incidence of hyperplasia (combined F0 and F1 males and females) was 0/94 controls, 71/94 (76%; 29 male/42 female) in the low-dose groups, 89/94 (95%; 37 male/52 female) in the mid-dose groups, and 94/94 in the high-dose groups. Benign neoplasms of the forestomach (squamous papilloma) occurred in 0/94 controls, 0/94 in the low-dose groups, 8/94 (9%) in the mid-dose groups, and 10/94 (11%) in the high-dose groups.

Hematological Effects. In mated female rats, orally gavaged with 0.2, 0.4, 0.5, 0.8, 1.0, 1.6, or 2.0 mg/kg/day of mustard gas acutely on gestation days 6 through 15, maternal hematocrit values were significantly reduced by 10.8% at 0.8 mg/kg/day and 5.4% at 1.0 and 2.0 mg/kg/day (DOA 1987b). While hematocrit at 1.6 mg/kg/day was reduced, the decrease was not significant.

A dose-related decrease in maternal hematocrit was reported following acute oral administration of mustard gas on gestation days 6 through 19 in inseminated female rabbits, 0.9, 2.8, and 9.1% at 0.4, 0.6, and 0.8 mg/kg/day, respectively, with statistical significance achieved only at the highest dose (DOA 1987b).

Serum protein concentrations were significantly decreased (8.3%) only in females in the highest-dose group of rats orally gavaged with 0.003, 0.01, 0.03, 0.1, or 0.3 mg/kg/day mustard gas for 5 days/week for 13 weeks (Sasser et al. 1996b). Blood urea nitrogen (BUN) and creatinine levels and serum glutamic

oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) activities in all treated groups were comparable with controls.

Endocrine Effects. Microscopic examination of adrenals from rats orally gavaged with 0.3 mg/kg/day mustard gas 5 days/week for 13 weeks revealed no lesions (Sasser et al. 1996b).

Dermal Effects. No dermal effects were observed in rats or rabbits acutely dosed with up to 2.5 mg/kg/day of mustard gas (DOA 1987b) or following longer exposures in rats orally gavaged with 0.08–0.5 mg/kg/day mustard gas 5 days/week for 10 weeks (Sasser et al. 1993), with 0.003–0.3 mg/kg/day mustard gas 5 days/week for 13 weeks (Sasser et al. 1996b), or with 0.03–0.4 mg/kg/day mustard gas for 18–21 weeks (Sasser et al. 1996a).

Ocular Effects. Ophthalmology evaluations of rats orally gavaged with 0.003–0.3 mg/kg/day mustard gas 5 days/week for 13 weeks revealed no abnormalities (Sasser et al. 1996b).

Body Weight Effects. In mated female rats orally gavaged with 0.2, 0.4, 0.5, 0.8, 1.0, 1.6, or 2.0 mg/kg/day of mustard gas acutely on gestation days 6 through 15, a significant dose-related decrease in maternal body weights was observed by gestation day 9 at 1.0 mg/kg/day (4.7–9.1%) and 2.0 mg/kg/day (6.5–16.0%) and by gestation day 12 at 0.5 mg/kg/day (4.1–6.6%) and 1.6 mg/kg/day (9.1–16.6%) (DOA 1987b). Extragestation weight gain was also dose-related with reductions of 10, 27, 25, 29, 38, 53, and 57% measured in 0.2, 0.4, 0.5, 0.8, 1.0, 1.6, and 2.0 mg/kg/day groups, respectively, compared to concurrent controls, with statistical significance achieved at $0.4 mg/kg/day.

Inseminated female rabbits orally gavaged with 0.4–2.5 mg/kg/day of mustard gas on gestation days 6 through 19, showed a significantly decreased maternal body weight at 0.8 mg/kg/day (after gestation day 10, 5 days of exposures, 7.9–10.5% decrease) and 2.0 mg/kg/day (after gestation day 14, 9 days of exposures, 12.0–18.3% decrease), but not at 1.0 mg/kg/day (DOA 1987b).

Females in the highest-dose group of rats orally gavaged with 0.003–0.3 mg/kg/day mustard gas 5 days/week for 13 weeks weighed significantly less than controls at week 4 and during the last 5 weeks of exposure (reduced >10%) (Sasser et al. 1996b). Males in the highest-dose group weighed significantly less than controls during 6 of the weeks in the weeks 3–12 of the study period (reduced by >8%). There was no indication of a dose response in body weight in lower dose groups.

In a two-generation reproductive study of mustard gas administered intragastrically at levels of 0.03–0.4 mg/kg/day, the body weights of the F0 exposed rats were not significantly different from controls; however, the growth rate of the high-dose males tended to decline after about 7 weeks of exposure (Sasser et al. 1996a). Body weight gain beginning 1 or 2 weeks after gavaging was started (approximately 20% for males and 15–24% for females) was significantly lower ($p < 0.05$) than control values in F1 rats of both sexes born to high-dose parents. No significant dose-response in body weight occurred at the lower doses.

Rats were orally gavaged with 0.08, 0.2, or 0.5 mg/kg/day mustard gas 5 days/week for 10 weeks (Sasser et al. 1993). While body weights of female rats in all treated groups were slightly less than controls, the differences were not significant. Male body weight was significantly reduced in the high-dose group beginning at week 2 (data present graphically), whereas the weights of the lower dose groups were comparable with control.

3.2.2.3 Immunological and Lymphoreticular Effects

In mated female rats, orally gavaged with 0.2–2.5 mg/kg/day of mustard gas acutely on gestation days 6 through 15, inflamed mesenteric lymph nodes were found in 0/34 control rats, and 0/8, 4/9 (44%), 11/25 (44%), 9/9 (100%), 16/25 (64%), 6/8 (75%), 17/30 (57%), and 3/3 (100%) at 0.2, 0.4, 0.5, 0.8, 1.0, 1.6, 2.0, and 2.5 mg/kg/day, respectively (DOA 1987b).

Enlarged Peyer's patches were found in inseminated female rabbits orally gavaged with 0.4–2.5 mg/kg/day of mustard gas on gestation days 6 through 19; however, incidence was not reported (DOA 1987b).

3.2.2.4 Neurological Effects

In rats, orally gavaged with 0.08, 0.2, or 0.5 mg/kg/day mustard gas, 5 days/week for 10 weeks, drooling following dosing was observed in the highest dose group (Sasser et al. 1993).

3.2.2.5 Reproductive Effects

In teratology studies in rats and rabbits, no significant increase in the number of resorptions was reported in pregnant animals of either species orally gavaged with 0.2–2.0 mg/kg/day of mustard gas acutely on gestation days 6 through 15 (in rabbits through gestation day 19) (DOA 1987b). In rats, at the highest dose of 2.0 mg/kg/day, a significant decrease in gravid uteri weight (16%) and sex ratio (46.2% males) occurred.

Microscopic examination of testes from rats orally gavaged with 0.3 mg/kg/day mustard gas 5 days/week for 13 weeks revealed no lesions (Sasser et al. 1996b).

Reproductive performance and fertility in male or female rats through two consecutive generations were studied following exposure to mustard gas via intragastric administration at levels of 0.03, 0.1, and 0.4 mg/kg/day (Sasser et al. 1996a). Microscopic examination of the reproductive organs revealed no evidence of treatment-related effects. The only statistically significant birth parameter difference between treated and control groups was an increase in the sex ratio (fraction of males) of the high-dose F0 offspring.

In a dominant lethal study of mustard gas, rats were orally gavaged with 0.08, 0.2, or 0.5 mg/kg/day mustard gas 5 days/week for 10 weeks (Sasser et al. 1993). In female dominant lethality experiments (treated or untreated males were mated with treated females), the overall mean pregnancy rate in treatment groups was 86%; treatment means ranged from 70 to 100%, with no significant differences between treatment groups. Reproductive performance indicators (number of live or dead implants, resorptions, and preimplantation losses) in treated female rats mated to treated or nontreated males were not significantly different from controls. In male dominant lethality experiments (treated males were mated with untreated females), the overall mean pregnancy rate in treatment groups was 91%; treatment means ranged from 65 to 100%, with no significant differences between treatment groups. There was no indication of a dose relationship with the number of live implants. In the highest exposure group, the mean number of total and early resorptions per litter was significantly greater than control during the 2nd and 3rd postexposure weeks. The number of total and late resorptions in the mid-dose group was also greater than controls during the 3rd postexposure week. Preimplantation losses in the mid- and high-dose groups were also significantly elevated during the 2nd postexposure week. High-dose male sperm morphology data at all postexposure sampling times, 0, 5, and 12 weeks, showed a statistically significant

decrease in the percentage of normal sperm. Blunthook and banana-shaped sperm heads were observed at 0, 5, and 12 weeks, whereas amorphous and short head abnormalities were observed only at 5 and 12 weeks. Overall, there was a total 2-fold increase in abnormal sperm heads in high-dose mustard gas-treated males. In summary, female fertility was not affected by these mustard gas exposures; however, a male dominant lethal effect was demonstrated at the mid and high doses of mustard gas.

3.2.2.6 Developmental Effects

Teratology studies were conducted in rats and rabbits by DOA (1987b). Rats were exposed to 0.5–2.0 mg/kg of mustard gas by gastric intubation from 6 to 15 days of gestation. Fetal body weight was significantly decreased (6–7%) from control in rat litters exposed to doses of 1.0 and 2.0 mg/kg/day; no clear dose-relation was evident. The sex ratio (percent males) was significantly lower than control at 2.0 mg/kg/day (46.2 vs. 51.0%). Placental weight was significantly reduced (8.4%) at 2.0 mg/kg/day. Supernumerary ribs were found in 9/299 (3%) fetuses of one litter in the 2.0 mg/kg/day group, while this anomaly was not found in any of the fetuses in the lower dose or control groups. The incidence of reduced ossification of the vertebrae and/or sternebrae in all treated groups was significantly higher than control when individual pup data were compared but not with litter comparisons, 42/272 (15%) in controls, 51/229 (22%) at 0.5 mg/kg/day, 76/315 (24%) at 1.0 mg/kg/day, and 72/299 (24%) at 2.0 mg/kg/day. All fetal effects in rats occurred at doses that also produced maternal toxicity. In rabbits exposed to 0.4–0.8 mg/kg of mustard gas between 6 and 19 days of gestation, the only effect of mustard gas on fetuses was a significant reduction in fetal body weight (38%), which occurred at 2.0 mg/kg/day, a dose that also produced maternal toxicity.

Developmental effects in male or female rats through two consecutive generations were studied following exposure to mustard gas via intragastric administration at levels of 0.03, 0.1, and 0.4 mg/kg/day (Sasser et al. 1996a). Although, not significantly different, litter weights and number of pups per litter tended to decrease in both F1 and F2 generations at the highest exposure level.

3.2.2.7 Cancer

No studies were located regarding cancer in humans or animals after oral exposure to mustard gas.

3.2.3 Dermal Exposure

3.2.3.1 Death

In France, two children died after a 40-year-old mustard gas shell accidentally exploded spraying the liquid onto their skin and clothing (Heully et al. 1956). Two fishermen died from handling mustard gas bombs disposed of in the Baltic Sea which became caught in their nets (Aasted et al. 1985; Jorgensen et al. 1985). Other surviving fishermen suffered skin lesions, erythema, blistering, and eye lesions. In humans, the LD_{50} for skin exposure is estimated to be 100 mg/kg (SBCCOM 1999). LD_{50} values in animals for mustard gas administered topically range from 9 to 100 mg/kg (Dacre et al. 1995). Of the species studied (rat, mouse, dog, rabbit, guinea pig, and goat), the rat was the most sensitive, with a dermal LD_{50} of 9 mg/kg.

3.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, musculoskeletal, renal, or body weight effects in humans or animals after dermal exposure to mustard gas.

Gastrointestinal Effects. Volunteers who were wearing respirators and who were exposed to unspecified levels of mustard gas vapors and liquids had skin burns, but also complained of nausea, vomiting, anorexia, abdominal pain, diarrhea, headache, and lassitude (Sinclair 1948). These signs could have been primary effects of the mustard gas on the rapidly dividing cells of the gastrointestinal epithelium, secondary effects from the skin burns, or psychological effects not related to the mustard gas exposure at all.

In a study designed to determine lethal dermal doses, rats stopped eating and drinking, had diarrhea, and lost weight prior to death (Young 1947).

Hematological Effects. Mustard gas was topically applied a single time at doses of 3.88, 7.75, or 15.5 mg/kg to the shaved backs of Balb/c mice (Venkateswaran et al. 1994). A reduction in lymphocytes was noted. Hematology revealed a significant dose-related increase in packed cell volume (10–16%). The increase in hemoglobin concentration was also dose-related and significant at mid and high doses (13–19%).

Hepatic Effects. Mustard gas was topically applied a single time at doses of 3.88, 7.75, or 15.5 mg/kg to the shaved backs of Balb/c mice (Venkateswaran et al. 1994). A dose-related decrease in liver weight was observed, with a significant reduction of 14% measured a the high dose.

A single dose of 51.3 mg/kg (1 LD_{50}) of neat mustard gas was applied to the hair-clipped backs of male guinea pigs (Chauhan and Murty 1997). At 24 hours after exposure, microscopic examinations of the liver revealed fatty degeneration accompanied by infiltration with red blood cells, lipidolysis, and distortion of cell structure. At 3 days postexposure, infiltration with macrophages was observed in addition to the above alterations. Liver injury was also indicated by increases in blood enzymes, glutamic oxaloacetic transaminase (GOT), and glutamic pyruvate transaminase (GPT). Both enzymes increased after exposure reaching maximum levels of nearly twice control values at 3 days, and returned toward normal levels at 6 days postexposure. The GOT recovery was slower than GPT as the 6-day level, while submaximal, was still significantly elevated (33%) above control.

Endocrine Effects. Mustard gas was topically applied a single time at doses of 3.88, 7.75, or 15.5 mg/kg to the shaved backs of Balb/c mice (Venkateswaran et al. 1994). A dose-related increase in adrenal weight was observed, significant at the mid and high doses (25–57%).

Dermal Effects. The severity of cutaneous injury is dose-dependent and is directly related to the mustard gas alkylation levels in skin (Papirmeister 1993). Vesication and acute tissue injury only occur at mustard gas alkylation levels much higher than those needed to produce genotoxic effects. Tissue injury does not develop when low, therapeutically effective doses of mustard gas are used to control the hyperproliferation of psoratic keratinocytes.

Mustard gas is more harmful to the skin on hot, humid days, or in tropical climates (Sulzberger et al. 1947). SBCCOM (1999) reports a maximum safe Ct of 5 mg-minute/m^3 for human skin exposure. The ICt_{50} (estimated concentration-exposure time period product incapacitating to 50% of exposed individuals) for human skin exposure is dependent on temperature, 2,000 mg-minute/m^3 at 70–80 EF (humid environment) and 1,000 mg-minute/m^3 at 90 EF (dry environment) (SBCCOM 1999).

When mustard gas gets on human skin, it causes erythema, itching, and blisters. These reactions are usually delayed by at least several hours, up to 48 hours (Jakubowski et al. 2000; Renshaw 1946; Smith et al. 1919). Australian soldiers, who were wearing respirators, volunteered to be exposed to skin contact with mustard gas during World War I. They had erythema on the exposed areas, and skin burns on the genitalia (Sinclair 1948, 1950). They also suffered from nausea and vomiting, but this may be secondary to the skin burns. Other subjective complaints, such as headache and lassitude, could be secondary to the burns, primary effects of the mustard gas, or due to other causes altogether. Men who were exposed to mustard gas from leaking artillery shells picked up by fishing vessels off the coast of Denmark showed inflamed skin, blisters, eye irritation, and transient blindness (Wulf et al. 1985). Army volunteers exposed to mustard gas had skin burns, but no increased incidence of skin cancer or other systemic effects (NRC 1985).

There is a case report of an accidental exposure to mustard gas in the laboratory (Jakubowski et al. 2000). A chemist was measuring what was believed to be a completely innocuous liquid mixture using a liquid flashpoint tester. The apparatus, which was in an approved chemical laboratory hood, overheated, causing vigorous vapor and aerosol generation from the sample, to which the subject was exposed primarily by skin contact. Personal decontamination was delayed for 5–10 minutes after exposure. Subsequent analysis showed that the liquid contained more than 5% mustard gas. The subject noticed no effects until about 9 hours after exposure, at which time, burning was experienced on his arms, hands, face, and neck. He applied a topical anesthetic-antiseptic before bed and awoke the next morning with blisters on his hands and arms. Mustard gas was not detected in the blister fluid, but thiodiglycol, a metabolite of mustard gas, was detected in his urine for 13 days following exposure.

A review of the literature prior to 1950 indicates that drops containing 0.1% or more mustard gas can cause skin blisters on humans (Sulzberger et al. 1947). The amounts applied during these studies could not be well quantified. Humans show varying degrees of sensitivity to mustard gas (Renshaw 1946; Sulzberger et al. 1947). In particular, people with fair skin are more sensitive than those with dark skin. These reports also indicate that individuals with previous exposure are more sensitive to the dermal effects of mustard gas.

A group of patients, including a subgroup of 14 children and teenagers (9 boys, aged 9 months to 14 years; 5 girls, aged 13 months to 9 years), were examined in a hospital in Iran 18–24 hours following exposure to mustard gas from air bombs during the Iran-Iraq War (Momeni and Aminjavaheri 1994).

Cutaneous effects included erythema (in 94% of patients), itching (71%), bulla (71%), ulceration (64%), hyperpigmentation (50%), and hypopigmentation (21%). Burning sensation (in 71% of patients) and pain (36%) were also noted. Skin lesions first appeared 4–18 hours after exposure, accompanied by an itching and burning sensation, especially over the face and neck. Thereafter, the patients developed erythema and gradually, after 20–30 hours, blisters. Most of the lesions in children developed of the face (79%), followed by genital (43%), thoracic (21%), trunkal (14%), and axillar lesions (14%). No direct relation was found between sex of the individual and the site of the lesions. The time of onset of mustard gas manifestations in children was shorter (4–18 hours) and the severity of the lesions higher than in adults (8–24 hours), possibly due to more delicate skin and epithelial tissues. Genital lesions were less frequent in children and teenagers (42%) than adults (70%); however, even within the group of children, the incidence and severity of genital lesions increased with age. Other skin lesions had no apparent age-relation.

Mustard gas applied to the skin of rats produced local edema, which subsided after 3 days (Young 1947). In mice, rabbits, and guinea pigs, mustard gas produced vascular leakage, leukocytic infiltration, and slow death of basal epidermal cells; this damage reached its peak 1–3 days after application (Vogt et al. 1984; Chauhan et al. 1993a, 1993b, 1995, 1996). Healing occurred within 10 days. Suckling rats (which had not yet grown hair) developed inflammatory changes and epidermal thickening after dermal exposure to mustard gas for 1–15 minutes (McAdams 1956). This damage was evident 1–7 days postexposure. Blisters did not develop, but the basal cells were destroyed.

Mustard gas was topically applied a single time at doses of 3.88, 7.75, or 15.5 mg/kg to the shaved backs of Balb/c mice (Venkateswaran et al. 1994). Mild skin lesions first appeared on postexposure day 4. Lesions progressed to severe, with fluid loss, on postexposure day 7.

Ocular Effects. The eyes are more sensitive to mustard gas than the skin or respiratory tract. In humans, an ICt_{50} (estimated concentration-exposure time period product incapacitating to 50% of exposed individuals) and a maximum safe Ct for eye exposure are 200 and 2 mg-minute/m^3, respectively (SBCCOM 1999). The damage may vary from mild conjunctivitis to severe corneal involvement with dense opacification, ulceration, and vascularization. In men, mild reddening of the eyes, but no incapacitation, resulted at 70 mg-minute/m^3 (McNamara et al. 1975). At 90 mg-minute/m^3, the eyes were marginally incapacitated with grittiness, photophobia, lacrimation, discharges, and staining, all of which disappeared within 4 days after exposure. Eye pain and spasmodic blinking are other reported ocular

effects. A temporary loss of vision occurred in men exposed to 100 or 144 mg-minute/m^3. In seven men who were accidentally exposed to estimated doses of 200–300 mg-minute/m^3, severe to total impairment of vision resulted in all (McNamara et al. 1975). Eyes exposed to mustard gas can also result in delayed reactions that are manifested as delayed relapsing keratitis (Amalric et al. 1965; Dahl et al. 1985; Mann 1944).

A group of patients, including a subgroup of 14 children and teenagers (9 boys, aged 9 months to 14 years; 5 girls, aged 13 months to 9 years), were examined in a hospital in Iran 18–24 hours following exposure to mustard gas from air bombs during the Iran-Iraq War (Momeni and Aminjavaheri 1994). Ocular effects of conjunctivitis and photophobia were most prevalent, each occurring in 93% of the children, with lower incidences of edema of the eyelids (57%), closure of the eyes (43%), keratitis (43%), blepharospasm (43%), subconjunctival hemorrhage (14%), and corneal ulcer in one child (7%). Burning sensation (71%) and pain (36%) were also noted. The burning sensation in their eyes developed 3–4 hours after exposure and was followed by photophobia and conjunctivitis. Ocular effects had higher occurrences in children than in adults.

The eyes of dogs that were exposed to 0.1 mg/m^3 (0.015 ppm) of mustard gas for 16 weeks showed corneal opacity, vascularization, and granulation (McNamara et al. 1975). Similar results were reported by Winternitz (1920), who exposed dogs acutely to unspecified levels of mustard gas.

The retinas of rats sacrificed 24 hours after injected subcutaneous injection in the dorsal area with 10 µL of undiluted radiolabeled mustard gas showed edematous swelling of the inner layers. Cell degenerative changes included dense cytoplasm, enlarged mitochondria, and Golgi apparatus. Rats sacrificed at 48 hours after injection had highly disorganized and vacuolated outer segment membranes and the choroid vessels contained large clusters of activated platelets (Klain et al. 1991).

Mustard gas was topically applied a single time at doses of 3.88, 7.75, or 15.5 mg/kg to the shaved backs of Balb/c mice (16/group/dose) (Venkateswaran et al. 1994). Reduced food consumption was noted in the high-dose group. A progressive dose dependent fall in body weight beginning 3–5 days after exposure was found; the decrease was significant at the mid and high doses, 11 and 27%, respectively.

Guinea pigs treated with a single dose of 51.3 mg/kg (1 LD_{50}) of neat mustard gas applied to their hair-clipped backs showed a gradual loss of weight up to 35% on postexposure day 6 (Chauhan and Murty 1997).

3.2.3.3 Immunological and Lymphoreticular Effects

Mustard gas was topically applied a single time at doses of 3.88, 7.75, or 15.5 mg/kg to the shaved backs of Balb/c mice (16/group/dose) (Venkateswaran et al. 1994). Mustard gas produced a significant dose-related decrease in the weight of the spleen (12–59%), and peripheral (12–44%) and mesenteric lymph nodes (significant only at high dose, 18%). Incidence and severity of histological changes in the thymus and spleen were also dose-related. Spleen histopathology included hypocellularity, atrophy of the lymphoid follicles, degeneration of germinal centers, and red pulp infiltrated with macrophages. The cortex and medulla regions of the thymus showed atrophy and hypocellularity. Red blood cells replaced cortical thymocytes with severe atrophy. A significant dose-related decrease in the cellularity of the spleen (24–45%) was measured. A dose-related decrease in the cellularity of the thymus was also found, significant at the mid and high doses (36–42%).

Cameron et al. (1946), after observing damage to the cervical lymph nodes and lymphoid tissue throughout the body in rabbits and monkeys that had undergone tracheal cannulation and were exposed to nominal chamber concentrations of mustard gas ranging from 30 to 350 mg/m^3 (5–54 ppm), administered mustard gas to animal skin and observed identical changes to the lymph tissue, suggesting that lymphoid tissue damage may be due to systemic absorption. Only a general discussion, lacking experimental details, was reported.

3.2.3.4 Neurological Effects

Chronic and/or late neurological symptoms in the skin after exposure to sulfur mustard were studied in five patients exposed to mustard gas during battlefield operations in the Middle East and five fishermen accidentally exposed to sulfur mustard by pulling shells leaking the chemical agent aboard their fishing vessels. All 10 patients (100%) suffered from neuropathic pain or other deafferentation symptoms, suggesting persistent damage to the afferent nerve system as a frequent complication in persons exposed to mustard gas (Thomsen et al. 1998).

Guinea pigs treated with a single dose of 51.3 mg/kg (1 LD_{50}) of neat mustard gas applied to their hair-clipped backs became sedated 1 day after exposure (Chauhan and Murty 1997).

No studies were located regarding the following heath effects in humans or animals after dermal exposure to mustard gas:

3.2.3.5 Reproductive Effects

3.2.3.6 Developmental Effects

3.2.3.7 Cancer

Five cases of Bowen's disease (a type of skin cancer) were studied among 488 former workers of a Japanese poison gas factory (Inada et al. 1978). Workers were manufacturing mustard gas for 3–15 years and the diagnosis was made 31–41 years after exposure. These workers also suffered from acute dermatitis, conjunctivitis, bronchitis, and hyperkeratotic skin eruptions. The occurrence of Bowen's disease, Bowen's carcinoma, basal cell carcinomas, and carcinoma spinocellular has also been reported in survivors of the dismantling of the "Heeres-Munitionsanstalt St. Georgen" who were exposed to poisonous gases including mustard gas by skin contact and inhalation (Klehr 1984).

No studies were located regarding cancer in animals after dermal exposure to mustard gas, although animals exposed to mustard gas in the air could have also had skin exposure. Cancer in these animals is discussed in Section 3.2.1.7 above.

3.2.4 Other Routes of Exposure

Several animals studies indicate effects of mustard gas on the hemopoietic system following intravenous or subcutaneous administration of mustard gas. Single intravenous injection of 0.5 mg/kg of mustard gas in young male rats caused degenerative damage to the spleen, thymus, and bone marrow (Kindred 1947). This was also observed in rats, mice, rabbits, and dogs following a subcutaneous injection of 3 mg/kg of mustard gas (Graef et al. 1948). Within 12 hours of injection, granulocytosis was observed, followed by leukopenia. In addition to hemopoietic tissue damage, injury to the testes with inhibition of spermatogenesis were also observed. Subcutaneous injection of 0.625 mg/kg of mustard gas in male rats caused injury to the thymus (Cataline et al. 1971). An intraperitoneal injection of 15 mg/kg of mustard

gas depressed the activity of bone marrow cells of the femur in mice (Friedberg et al. 1983). Parental administration of mustard gas to laboratory animals resulted in death due to systemic intoxication, with little or no involvement of the eyes or skin. Damage to the lungs is seen with intravenous administration, but not other parenteral routes (Anslow and Houch 1946).

A significant dose-related reduction in spleen cell number was measured in mice 7 days after intraperitoneal injection with mustard gas (23% at 5 mg/kg and 49% at 10 mg/kg) (Coutelier et al. 1991). Sv female mice (5–9/group) were injected intraperitoneally with a single dose of 2, 5, or 10 mg/kg mustard gas (>90% purity) in a 1% isopropanol solution in saline. A 26% increase in spleen T-lymphocytes and a 44% decrease in B-lymphocytes was measured 7 days following injection with 10 mg/kg of mustard gas. B- and T-lymphocyte function, as assayed by *in vitro* thymidine incorporation and/or immunoglobulin secretion, was not significantly affected by mustard gas.

Mustard gas, administered to guinea pigs by intratracheal injection, induced a 3-fold increase in respiratory system resistance, accompanied by a significant decrease in compliance (Calvet et al. 1993). Capsaicin-sensitive nerves do not have primary involvement in the acute respiratory effects of mustard gas as preteatment with capsaicin did not prevent acute effects. Fourteen days after exposure, substance P induced concentration-dependent bronchoconstriction in guinea pigs, and tracheal epithelium neutral endopeptidase (NEP), the main enzyme that degrades tachykinins, was reduced significantly (40%) from the control level. While hyper-responsiveness to substance P has been attributed to a decrease in the tracheal activity of NEP and corresponding increase in tachykinins, this hypothesis was not upheld, as pretreatment with phosphoramidon, a NEP inhibitor, only increased mustard gas-induce hypersensitivity to substance P. Phosphoramidon administered prior to vehicle control ethanol also increased sensitivity to substance P.

3.3 GENOTOXICITY

Low doses of mustard gas can inhibit cell division due to its ability to cross-link complementary strands of DNA or produce mutagenesis, which may be caused by replication errors or misrepair (Papirmeister 1993). DNA is the most functionally sensitive target of mustard gas in cells. Men who were exposed to mustard gas from leaking shells picked up by fishing vessels showed increased sister chromatid exchanges in their lymphocytes (Wulf et al. 1985). However, the offspring of workers exposed to mustard gas in a Japanese factory showed no increases in diseases that would be indicative of genetic

damage (Yamakido et al. 1985). Mustard gas induced dose-related interstrand cross-links in the DNA of rat epidermal keratinocytes in primary monolayer cultures, synchronized at the G1/S boundary or in the G1 phase of the cell cycle (Lin et al. 1996a). At 24 hours postexposure, the level of cross-links in cells exposed at the G1 phase had not decreased significantly and was still dose-dependent. However, at 24-hours postexposure, cells exposed in the G1 phase showed a major decrease in cross-links.

DNA extracted from white blood cells of human blood and exposed to [14C]-labeled mustard gas *in vitro* was shown to contain the DNA adduct 7-(2-hydroxyethylthio-ethyl) guanine (Ludlum et al. 1994). Mustard gas alkylation has been shown to induce effects on transcriptional processes (Masta et al. 1996). Gel mobility shift analysis showed that mustard gas alkylation of the lac UV5 promoter increased the stability of the promoter-RNA polymerase binary complex. Following formation of the initiation complex and addition of elongation nucleotides, approximately 45% of the RNA polymerase in the initiated complex remained associated with the alkylated promoter, compared to only 7% remaining associated with the unalkylated promoter. For the RNA polymerase able to escape the initiation complex, mustard gas alkylation of the DNA template resulted in the production of truncated transcripts. Analysis of the truncated transcripts revealed that mustard gas alkylates DNA preferentially at 5'-AA, 5'-GG, and 5'-GNC sequences on the DNA template strand.

Mustard gas has been shown to affect the cell cycle and DNA synthesis in epidermal basal keratinocytes. When primary keratinocytes were exposed to mustard gas in different phases of the cell cycle, cells in the S phase were more sensitive to mustard gas than cells in the other phases (Lin et al. 1996b). Keratinocytes exposed to 1 µM mustard gas at the G1/S boundary exhibited a prolongation of the S phase and a block in the G2 phase. When these cells were exposed to 10 or 50 µM mustard gas, they did not enter the S phase for up to 12 hours and the incorporation of thymidine into DNA was inhibited, suggesting that the blocks in the G2 and G1 phases relate to the cytotoxic effect of mustard gas.

Mustard gas at concentrations of 0.5 and 0.1 mM produced single strand breaks in bacteriophage lambda DNA (Venkateswaran et al. 1994), which were prevented by the presence of magnesium ions in the reaction mixture. The authors proposed that the degradation of lambda DNA by its interaction with mustard gas may be caused by the breakage of phosphodiester backbone of DNA via the formation of an intermediate phosphotriester bond.

Mustard gas-induced DNA damage in primary monolayer cultures of rat cutaneous keratinocytes was assessed with the nucleoid sedimentation assay (Ribeiro et al. 1991). Within 1 hour of exposure to as little as 0.1 µM mustard gas, the structural integrity of cellular DNA was compromised. The gross structural integrity of the DNA in cells exposed to as much as 5 µM mustard gas was completely restored within the first 22 hours following the exposure. However, this repair process appeared to be inefficient, since a depression of thymidine incorporation into DNA and a significant loss of DNA were exhibited in exposed cultures as long as 72 hours after the initial exposure.

Fan and Bernstein (1991) evaluated the possible effects of mustard gas on the repair of mismatched bases in the DNA of African green monkey kidney (AGMK) cells transfected with heteroduplex (ht) DNA, formed between two temperature-sensitive mutants of SV40 virus, tsA239 and tsA255, each having a different point mutation in the gene for large T antigen. In order for the cells to produce wild type SV40 DNA at a nonpermissive temperature, repair of at least one of the two mismatches in the DNA had to occur. As the concentration of mustard gas was increased, a proportionally longer delay in the appearance of wild type DNA was observed in treated cells transfected with ht DNA as compared with cultures exposed to solvent alone and then transfected with ht DNA. This effect did not occur in mustard gas exposed AGMK cells transfected with wild type DNA, suggesting that mustard gas does affect mismatched base repair.

A variety of *in vitro* assays, summarized in Table 3-3, provide positive genotoxicity results. These data support the few human data on *in vivo* exposures to this compound. The *in vitro* data from both prokaryotic organisms (*Salmonella typhimurium* and *Escherichia coli*) and eukaryotic organisms (HeLa cells, mouse lymphoma, mouse L cells, rat lymphosarcoma) all support a mechanism of DNA alkylation leading to cross-link formation, inhibition of DNA synthesis and repair, point mutation, and chromosome and chromatid aberration formation.

There are also data from *Drosophila* experiments in which sulfur mustard was injected into male flies, leading to sex-linked lethal mutations and point mutations at one of the loci affecting bristle formation (Auerbach 1946; Fahmy and Fahmy 1971, 1972). Mustard gas has also been shown to be a micronucleus-inducing agent to the mouse bone marrow (Ashby et al. 1991). All of these data are consistent with this agent being a powerful genotoxicant, which supports the recognized carcinogenicity of mustard gas.

Table 3-3. Genotoxicity of Mustard Gas *In Vitro*

Species (test system)	End point	Results: With activation	Results: Without activation	Reference
Prokaryotic organisms:				
Escherichia coli	DNA interstrand crosslinks	+	No data	Venitt 1968
E. coli	DNA recombination repair inhibition	+	+	Ichinotsubo et al. 1977
Salmonella typhimurium	Gene mutation	+	+	Ichinotsubo et al. 1977
S. typhimurium	Gene mutation	+	+	Ashby et al. 1991
Eukaryotic organisms:				
Fungi:				
Saccharomyces cerevisiae	DNA alkylation	+	No data	Kircher and Brendel 1983
Human HeLa cells in culture	DNA crosslinking	+	No data	Ball and Roberts 1971/72
Mouse lymphoma cells	Gene mutation	+	No data	Capizzi et al. 1974
Mouse lymphoma cells	Chromosomal and chromatid aberrations	+	No data	Scott et al. 1974
Rat lymphosarcoma cells	Chromosomal and chromatid aberrations	+	No data	Scott et al. 1974
Rat lymphosarcoma cells	DNA replication repair inhibition	+	No data	Scott et al. 1974
Mouse fibroblasts, L-strain	Inhibition of DNA synthesis	+	No data	Walker and Thatcher 1968

+ = positive result; DNA = deoxyribonucleic acid

Transcription, translation, and enzyme catalysis, cellular activities that are dependent on biological entities of much lower molecular size than chromosomal DNA, are much less sensitive to mustard gas (Papirmeister 1993). Thus, cells that are prevented from synthesizing DNA continue to generate energy and synthesize RNA and protein. As a result of this unbalanced metabolism, cells may enlarge, differentiate, or be induced to synthesize high levels of certain proteins. While some of these proteins may protect cells, others may hasten cell death.

Vesication and acute tissue injury only occur at mustard gas alkylation levels much higher than those needed to produce genotoxic effects. Tissue injury does not develop when low, therapeutically effective doses of mustard gas are used to control the hyperproliferation of psoratic keratinocytes. Therefore, it is likely that additional mechanisms other those related to genotoxicity are responsible for acute toxicity of mustard gas.

3.4 TOXICOKINETICS

There is a substantial toxicokinetic database for intravenous and intraperitoneal routes of mustard gas exposure in animals. While these data are useful, there is evidence to suggest that this information does not mimic the scenario resulting from field or accidental conditions that expose humans to mustard gas by absorption from the skin, or by the lung or eyes. Mustard gas tissue distribution data from an Iranian soldier who died 7 days after inhalation and/or dermal exposure to mustard gas indicated distribution: brain > kidney > liver > spleen > lung (Drasch et al. 1987), whereas radiolabel concentration data in rats 4 days after an intravenous injection of radiolabeled mustard gas indicate a different distribution pattern to these organs: kidney > lung > liver > spleen > brain (Maisonneuve et al. 1994). While the difference could be due to measurement methods, species variations, or postexposure time, the route of exposure appears to be a significant toxicokinetic factor.

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

Since mustard gas can be found in human tissues following exposure through the air, it can apparently be absorbed through the lungs or skin (Drasch et al. 1987). Analyses of the blood of hairless guinea pigs

after 8-minute nose-only exposure to 300 mg/m^3 (46 ppm) of mustard gas indicated that the concentration of mustard gas in blood peaked within 5 minutes after exposure (Langenberg et al. 1998).

In rabbits and monkeys that had undergone tracheal cannulation and were exposed to nominal chamber concentrations of 40, 100, and 500 mg/m^3 of mustard gas, about 15% of the dose was recovered, indicating that 85% was absorbed through the mucous membrane of the nose (Cameron et al. 1946).

3.4.1.2 Oral Exposure

No studies were located regarding absorption in humans or animals after oral exposure to mustard gas.

3.4.1.3 Dermal Exposure

Since mustard gas can be found in bodily tissues of humans following exposure through the air, it is apparently absorbed through the lungs or through the skin (Drasch et al. 1987). When applied to human skin, most of the mustard gas evaporates (Smith et al. 1919). Some of the vapors can be absorbed into the skin, with the majority of this being absorbed into the blood stream (Cullumbine 1946, 1947; Nagy et al. 1946; Renshaw 1946). Renshaw (1946) reported that 80% of unoccluded, topically-applied mustard gas evaporates from the skin and the remaining fraction penetrates the skin. This finding has been confirmed in studies of human foreskin grafted onto athymic mice (Papirmeister et al. 1984a, 1984b).

The absorption of mustard gas through the cornea was demonstrated in guinea pigs (Klain et al. 1991). Following 30 minutes after a single topical application of 5 µL of radiolabeled mustard gas to the cornea of guinea pigs, radioactivity was detected in kidney, liver, lung, adipose tissue, adrenals, plasma, and muscle.

Hambrook et al. (1993) reported that after a 6-hour cutaneous exposure with occlusion, >90% of applied dose was absorbed in rat skin. The initial rate of uptake, within 60 minutes of loading, increased linearly with applied dosage in the range of 3–605 µg/cm^2 (0.2–3.8 µmol/cm^2), and reached a maximum of approximately 7 µg/cm^2/minute (0.044 µmol/cm^2/minute) at a dosage of 955 µg/cm^2 (6 µmol/cm^2) (Hambrook et al. 1993). A range of skin-retention fractions from 10 to 50% have been reported (Cullumbine 1947; Hambrook et al. 1992; Renshaw 1946), while the remaining mustard gas is absorbed systemically. The rate of penetration of mustard gas into human skin was estimated in the range of

1–4 µg/cm^2/minute (0.006–0.025 µmol/cm^2/minute) (Renshaw 1946). Skin penetration of mustard gas is proportional to its temperature (Nagy et al. 1946). Some authors have suggested that the mustard gas is absorbed into the skin by passing into the sweat glands (Smith et al. 1919).

3.4.1.4 Other Routes of Exposure

No studies were located regarding absorption in humans or animals after exposure to mustard gas by routes other than inhalation and dermal.

3.4.2 Distribution

3.4.2.1 Inhalation Exposure

Analyses of body fluids and tissues of an Iranian soldier who died 7 days after exposure to mustard gas (by inhalation and/or dermal routes) indicated that mustard gas was distributed to cerebrospinal fluid and, in order of decreasing concentrations, fat (from thigh), brain, abdominal skin, kidney, muscle, liver, spleen, and lung (Drasch et al. 1987). No mustard gas was found in the urine of this patient. Analyses of the blood of hairless guinea pigs after 8-minute nose-only exposure to 300 mg/m^3 (46 ppm; 2,400 mg-minute/m^3) of mustard gas indicated that the concentration of mustard gas in blood peaked within 5 minutes after exposure, dropped to about 50% of peak at 30 minutes, and gradually increased again to about 60% of peak concentration at 4 hours after exposure (Langenberg et al. 1998). Evidence of tissue mustard gas DNA adducts in hairless guinea pigs at 4 hours after 5-minute nose-only exposure to 160 mg/m^3 (25 ppm; 800 mg-minute/m^3) of mustard gas indicates absorption and/or distribution to nasal epithelium, nasopharynx, larynx, carina, and lung (Langenberg et al. 1998). Mustard gas DNA adducts found in the lung, spleen, and bone marrow in the same species after 8-minute nose-only exposure to 300 mg/m^3 (46 ppm; 2,400 mg-minute/m^3) of mustard gas indicates distribution to these tissues (Langenberg et al. 1998).

3.4.2.2 Oral Exposure

No studies were located regarding distribution in humans or animals after oral exposure to mustard gas.

3.4.2.3 Dermal Exposure

Analyses of body fluids and tissues of an Iranian soldier who died 7 days after exposure to mustard gas (by inhalation and/or dermal routes) indicated that mustard gas was distributed to cerebrospinal fluid and, in order of decreasing concentrations, fat (from thigh), brain, abdominal skin, kidney, muscle, liver, spleen, and lung (Drasch et al. 1987). No mustard gas was found in the urine of this patient. Older reports have stated that mustard gas is distributed to most tissues in humans (Cullumbine 1947). Hambrook et al. (1993) reported that after a 6-hour cutaneous exposure to radiolabeled mustard gas with occlusion, 10–23% of absorbed radiolabel dose was retained in rat skin, with range of 3–7% detected in blood. At the end of the 6-hour application, when the level of radiolabel in the blood reached a maximum, greater than 90% of the red cell radiolabel activity was found in the cell contents, with the remaining in the red cell membranes.

In guinea pigs, following a single topical application of 5 µL of radiolabeled mustard gas to the cornea, radioactivity at 30 minutes after application, as expressed per unit weight, was greatest in the kidney followed by liver, lung, adipose tissue, adrenals, plasma, and muscle (Klain et al. 1991). At 2 and 5 hours postadministration, the greatest radioactivity per unit weight was again measured in the kidney, whereas the level in the plasma increased and that in the liver and lung decreased with postadministration time. Expressed per organ, the liver contained the highest level of radioactivity, followed by the kidney and lung. At 30 minutes postapplication, radioactivity was widely distributed in the guinea pig eye; the choroid/sclera portion contained the highest level followed by cornea, retina, and lens. Low levels were also detected in the aqueous and vitreous humors. At 5 hours, the only eye compartment in which the radioactivity level had decreased significantly from the 30 minutes value was the choroid/sclera portion.

3.4.2.4 Other Routes of Exposure

Boursnell et al. (1946) observed significant radioactivity levels in the kidney, lung, and liver of rabbits after intravenous injection of 5 mg/kg of radiolabeled mustard gas. Lower levels of radioactivity were also detected in bone marrow, spleen, stomach wall, duodenal wall, brain, heart, muscle, skin, and thyroid. Six hours after intravenous injection of 8.2 mg/kg of radiolabeled mustard gas into male hairless guinea pigs, radiolabel was distributed in decreasing concentrations to the bone marrow, liver, spleen, blood, and lung (Langenberg et al. 1998). In the rat, mustard gas is quickly and widely distributed (Maisonneuve et al. 1993, 1994; Zhang and Wu 1987). Maisonneuve et al. (1993) reported a distribution

volume of 74.4 L/kg and a half-life of 5.6 minutes following intravenous bolus administration of 10 mg/kg (3 LD_{50}) of mustard gas in the rat. The concentration of unchanged mustard gas in the blood decreased quickly in the first half hour, but low levels were detectable up to 8 hours after administration. The large volume of distribution, greater than the volume of body water, suggests a wide distribution of mustard gas throughout the animal. A quantitative distribution analysis was performed by Maisonneuve et al. (1994) in rats intravenously injected with radiolabeled mustard gas. Radioactivity was detected in blood, plasma, kidney, liver, intestine and stomach, heart, lung, brain, spleen, eyes, testicle, and adrenal gland. From 10 minutes to 6 hours after administration, the liver and kidney had higher radiolabel concentrations than the blood. The organs with the lowest levels of radioactivity were the brain, spleen, eye, and testicle. Maximum radioactivity levels in the organs were reached between 2 and 3 hours after injection. Total radioactivity in any organ did not exceed 4% of the administered dose. Most of the administered radioactivity was recovered in the muscle; 51% measured in muscle at 5 minutes, 36% in muscle at 3 hours, 3% in fat at 35 minutes, 10% in skin at 35 minutes (radioactivity peaked in fat and skin at 35 minutes).

In vitro studies of plasma and red blood cells treated with radiolabeled mustard gas indicate a high affinity of mustard gas toward red blood cells (Maisonneuve et al. 1993). The mean equilibrium red blood cell/plasma radiolabel concentration ratios for treatments with 4 and 400 µg/mL radiolabeled mustard gas were 2.12 and 4.15, respectively.

Radiolabeled mustard gas administered in rats to the femoral or jugular veins resulted in different organ distribution patterns. Subsequent to femoral vein injection, the injected leg was a site of significant mustard gas distribution, whereas jugular vein injection did not result in significant accumulation in the lung (Maisonneuve et al. 1994). The heart, lung, brain, and spleen received greater proportionate shares of radioactivity 35 minutes after jugular vein injection compared to femoral vein administration.

In the eyes of rats examined 4 hours after subcutaneous injection in the dorsal area with 10 µL of undiluted radiolabeled mustard gas, the largest amount of radioactivity was found in the pooled aqueous and vitreous humors (70%), followed by retina (12%), choroid/sclera (8%), lens (6%), and cornea (3%) (Klain et al. 1991).

3.4.3 Metabolism

The metabolism of mustard gas has not been studied extensively. Metabolic pathways including direct alkylation reactions, glutathione reactions, hydrolysis, and oxidation are presumed based on the finding of mustard gas DNA adducts in tissues and the identification of urinary products.

3.4.3.1 Inhalation Exposure

Mustard gas DNA adducts were found in the nasal epithelium, nasopharynx, larynx, carina, lung, spleen, and bone marrow of hairless guinea pigs after nose-only exposure to mustard gas (Langenberg et al. 1998).

3.4.3.2 Oral Exposure

No studies were located regarding metabolism in humans or animals after oral exposure to mustard gas.

3.4.3.3 Dermal Exposure

Studies of casualties of the Iran-Iraq War have identified significant amounts of mustard gas metabolite, thiodyglycol, in human urine more than a week after mustard gas exposure (Wils et al. 1985, 1988). The presence of this urinary biotransformation product is consistent with findings in animal studies discussed below in which mustard gas was administered by alternate routes. Sandelowsky et al. (1992) reported the detection of mustard gas metabolite, 4-met-1-imid-thiodiglycol, in plasma and urine following dermal exposure of mustard gas in pigs.

3.4.3.4 Other Routes of Exposure

Rats and mice were injected intraperitoneally with radiolabeled mustard gas, bis-2-chloroethyl-sulfide-^{35}S (Davison et al. 1961). The metabolism of this substance is apparently largely due to glutathione reactions, hydrolysis, and oxidation, since the major urinary metabolites were glutathione-bis-2-chloroethyl sulfide conjugates (45% of total), thiodiglycol and conjugates (14%), and sulfone products (20%). Slightly different results were reported by Roberts and Warwick (1963), who found that at least 50% of the urinary metabolites in rats was a conjugated form of bis-cysteinyl-ethylsulphone.

Thiodiglycol accounted for 15–20% of the urinary radioactivity, and 10–15% was a sulfide. Black et al. (1992b) investigated the metabolism of mustard gas similarly in the rat. Many metabolites were present in the urine, nine of which were identified as thiodglycol sulphoxide, 1,1'-sulphonybis[2-(methyl-sulphinyl)ethane], 1-[S-(N-acetylcysteinyl)]-2-(ethenylsulphonyl)ethane, 1-methylsulphinyl-2-[2-(methylthio)ethylsulphonyl]ethane, two diastereoisomers of 1-[S-(N-acetylcysteinyl)]-2-(2-chloroethyl-sulphinyl)ethane, 1,1'-sulphinybis[2-chloroethane], 1,1'-sulphonybis[2-S(N-acetylcysteinyl)ethane], and 1-[S-(N-acetylcysteinyl)]-2-(2-chloroethylsulphonyl)ethane, allowing the construction of a putative metabolic pathway (Black et al. 1992b). Black et al. (1992b), while confirming the major metabolic transformations of Davison et al. (1961), identified thiodiglycol sulphoxide as the major urinary excretion product and not the initial hydrolysis product thiodiglycol. The finding of metabolites 1,1'-sulphonybis-[2-(methylsulphinyl)ethane] and 1-methylsulphinyl-2-[2-(methylthio)ethylsulphonyl]ethane revealed a pathway for the degradation of glutathione conjugates formed via the action of enzyme β-lyase on cysteine conjugates. Renal β-lyase metabolism has also been implicated in the formation of nephrotoxic intermediates from halogenated alkenes.

A comparison of unchanged radiolabeled mustard gas and total radiolabel concentrations in the blood following intravenous bolus administration of radiolabeled mustard gas in rats indicated that much of the mustard gas is metabolized with a half-hour after administration (Maisonneuve et al. 1993).

3.4.4 Elimination and Excretion

Urinary excretion is the primary route of elimination for mustard gas and/or its metabolites.

3.4.4.1 Inhalation Exposure

People who were exposed to mustard gas during the Iran-Iraq War could have absorbed the material through the lungs or through the skin. One of the breakdown products of mustard gas, thiodiglycol, has been detected in the urine of these people (Wils et al. 1985). These authors also report that thiodiglycol is found in unexposed persons and cannot be used to determine the exact level of mustard gas exposure, although it could possibly be used to show large exposures. Unmetabolized mustard gas was also found in urine and feces samples from two Iran-Iraq War victims (Heyndrickx and Heyndrickx 1984; Mandl and Freilinger 1984; Pauser et al. 1984; Vycudilik 1985). No studies regarding animal excretion data from inhalation exposure are available.

3.4.4.2 Oral Exposure

No studies were located regarding excretion in humans or animals after oral exposure to mustard gas.

3.4.4.3 Dermal Exposure

People who were exposed to mustard gas during the Iran-Iraq War could have absorbed the material through the lungs or through the skin. One of the breakdown products of mustard gas, thiodiglycol, has been detected in the urine of these people (Wils et al. 1985). These authors also report that thiodiglycol is found in unexposed persons, and cannot be used to determine level of mustard gas exposure.

Jakubowski et al. (2000) measured the excretion of thiodiglycol in human urine following an accidental mustard gas exposure. In contrast to Wils et al. (1985), detectable levels of thiodiglycol in urine were measured for 13 days after exposure. The patient's urine was random sampled for 6 months after exposure and no further thiodiglycol elimination was detected. Maximum thiodiglycol excretion was seen on postexposure day 4. First-order elimination kinetics were observed and the half-life of thiodiglycol elimination was estimated to be 1.2 days.

Hambrook et al. (1992) reported that in the rat, following a 6-hour cutaneous exposure to radiolabeled mustard gas with occlusion, the urinary excretion of radiolabel had a half-life of 1.4 days; the half-life of excretion in feces, which varied slightly with dose, was approximately 1.6 days. Most of the radioactivity was found in the urine. Most of the dose was eliminated by 3 days; however, urinary excretion of radiolabel continued for greater than 3 months.

3.4.4.4 Other Routes of Exposure

Two terminal cancer patients were injected intravenously with radiolabeled mustard gas dissolved in ethanol (Davison et al. 1961). Several minutes after administration, 80–90% of the radioactivity was cleared from the blood. The residual level remained constant in both plasma and cells for at least 2 days, suggesting binding to some blood constituent. Excretion of 21% of the radioactivity in the urine occurred within 3 days. The metabolites found in the liver were the same as those in rats, suggesting that human metabolism is similar to rat metabolism for this chemical.

The major route of elimination of radioactivity in the rat, after intravenous injection of radiolabeled mustard gas is by the kidney (Boursnell et al. 1946; Davison et al. 1961; Hambrook et al. 1992; Maisonneuve et al. 1993). Maisonneuve et al. (1993) reported a blood clearance of 21 L/hours-kg and elimination half-life of 3.59 hours from blood concentration data following intravenous bolus administration of 10 mg/kg (3 LD_{50}) of radiolabeled mustard gas in the rat. Similarly to that found in humans, a residual constant level of radioactivity was found in blood for 2 days after exposure; a slight increase in the residual level was observed between 2 and 4 days. The largest overall recovery of radioactivity was in urine, with about 65% of the administered dose excreted during 24 hours and 80% during 96 hours, a much higher percentage than that reported for humans (Davison et al. 1961). Fecal excretion accounted for <3% of the administered dose during 96 hours (Maisonneuve et al. 1993).

Rats and mice that were injected intraperitoneally with radiolabeled mustard gas excreted 50–78% of the radioactivity within 1 day and 90% within 3–5 days in the urine (Black et al. 1992a; Davison et al. 1961; Roberts and Warwick 1963; Smith et al. 1958). Twelve hours after intraperitoneal injection, 6% was excreted in the feces and 0.05% in the expired air (Davison et al. 1961).

Hambrook et al. (1992) measured the excretion of radiolabel in urine and feces in the rat following intravenous or intraperitoneal injection of radiolabeled mustard gas. The half-life varied little with dose, route, or excretion type and an average value of 1.4 days was reported. The pattern of excretion was similar after intraperitoneal and intravenous injections. Most of the dose was eliminated by 3 days; however, urinary excretion of radiolabel continued for greater than 3 months. About 65% of absorbed radiolabel was found in the urine and 11% in feces within 24 hours after administration.

3.4.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

Physiologically based pharmacokinetic (PBPK) models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic end points.

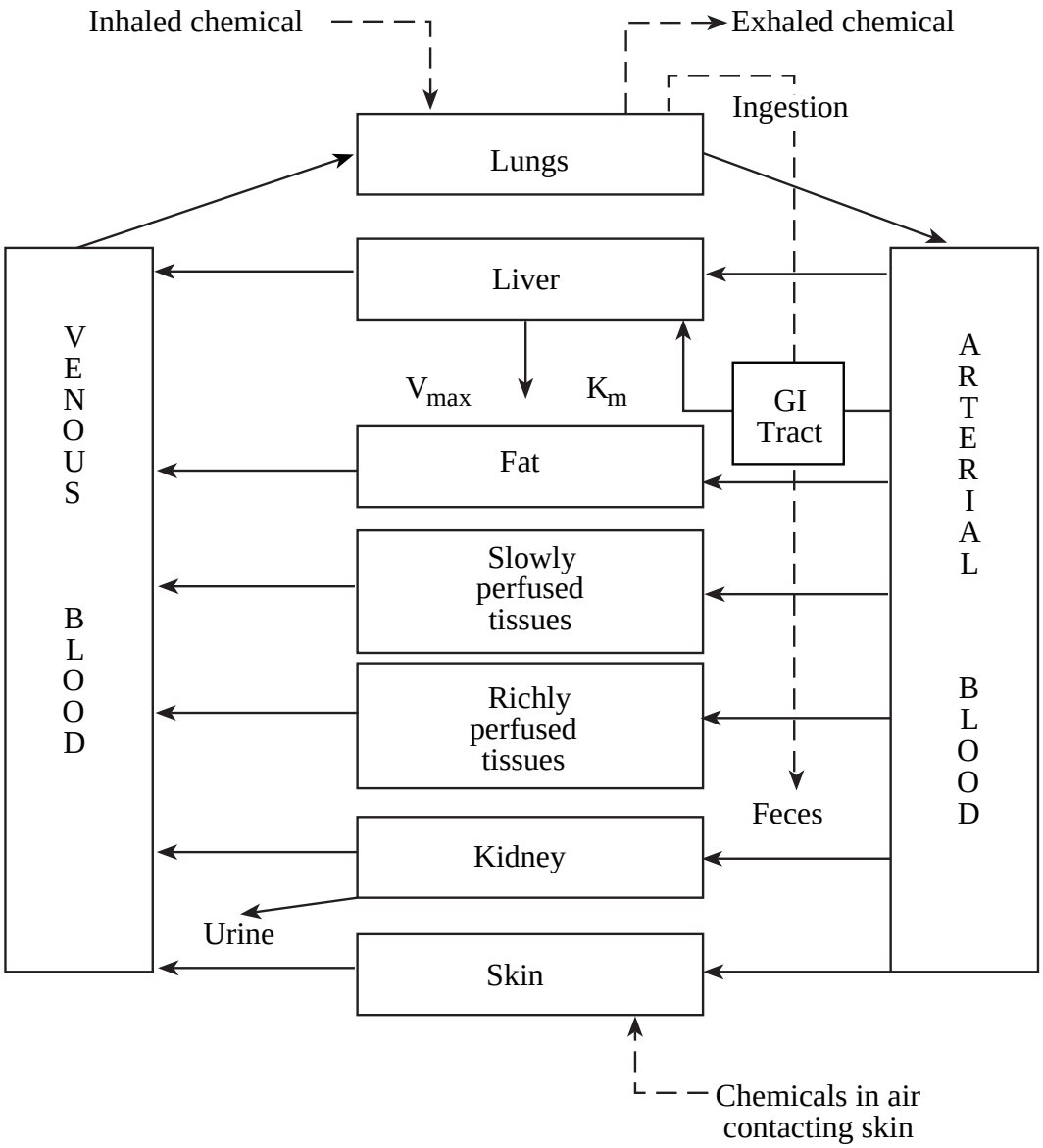
PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen et al. 1987; Andersen and Krishnan 1994). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parametrization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) is adequately described, however, this simplification is desirable because data are often unavailable for many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where doses were higher or were administered in different species. Figure 3-3 shows a conceptualized representation of a PBPK model.

Figure 3-3. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



Note: This is a conceptual representation of a physiologically based pharmacokinetic (PBPK) model for a hypothetical chemical substance. The chemical substance is shown to be absorbed via the skin, by inhalation, or by ingestion, metabolized in the liver, and excreted in the urine or by exhalation.

Source: adapted from Krishnan et al. 1994

No PBPK models exist for mustard gas. Toxicokinetic information is insufficient for modeling.

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

Absorption. Mustard gas is slightly soluble in water, but both the liquid and vapor forms are readily soluble in oils, fats, alcohol, and organic solvents. Because of its high lipid solubility, mustard gas quickly penetrates the lipid cell membrane.

Distribution. It has been estimated that about 12% of dermally absorbed mustard gas reacts with components in skin and the remainder is distributed in greatest proportion to the kidney and fairly evenly throughout the rest of the body as unreacted mustard gas or hydrolyzed mustard gas. In studies with radiolabeled mustard gas, tissue radioactivity levels increased as early as 5 minutes after intravenous injection and 15 minutes after percutaneous administration.

Metabolism. Mustard gas is presumed to be biotransformed by direct alkylation reactions, glutathione reactions, hydrolysis, and oxidation are based on the finding of mustard gas DNA adducts in tissues and the identification of urinary products.

Excretion. Urinary excretion is the primary route of elimination for mustard gas and/or its metabolites. In humans, elimination follows first-order kinetics and the half-life of thiodiglycol elimination is estimated to be 1.2 days (Jakubowski et al. 2000).

3.5.2 Mechanisms of Toxicity

At the cellular level, mustard gas interacts with nucleophiles on the cell membrane, at intracellular sites, and with nucleic acids (Papirmeister et al. 1991). While mustard gas is able to alkylate DNA, RNA, and proteins affecting a variety of cell functions, including altering proteins that have been coded by alkylated RNA and structurally altering cell membranes, DNA is the most functionally sensitive target of mustard gas in cells. Low doses of mustard gas can inhibit cell division due to its ability to cross-link complementary strands of DNA (Papirmeister 1993). Transcription, translation, and enzyme catalysis, cellular activities that are dependent on biological entities of much lower molecular size than

chromosomal DNA, are much less sensitive to mustard gas. Thus, cells that are prevented from synthesizing DNA continue to generate energy and synthesize RNA and proteins. As a result of this unbalanced metabolism, cells may enlarge, differentiate, or be induced to synthesize high levels of certain proteins. While some of these proteins may protect cells, others may hasten cell death.

Mechanisms of the toxicity of mustard gas have been postulated, but none have been demonstrated with certainty (Papirmeister 1993, 1994; Somani and Babu 1989; Whitfield 1987). As discussed in Section 3.3, it appears that different mechanisms are responsible for the acute and delayed effects of mustard gas and that additional mechanisms besides genotoxicity mechanisms are responsible for mustard gas vesication since acute skin injury develops at a time much earlier than expected from genotoxic effects. Also, tissue injury does not develop when low, therapeutically effective doses of mustard gas are used to control the hyperproliferation of psoratic keratinocytes. While the mechanisms of mustard gas toxicity are not currently fully understood, one hypothesis for mustard gas cytotoxicity involves poly(adenosine diphosphoribose) polymerase (PADPRP). The following mechanism for skin damage has been proposed: mustard gas alkylates DNA, which causes DNA breaks; numerous mustard gas-induced DNA strand breaks cause activation of nuclear repair enzyme PADPRP. This causes cellular depletion of nicotinamide adenine dinucleotide (NAD^+), which decreases glycolysis, which leads to protease release and cellular injury. Dermal-epidermal separation and blister formation may involve the fragmentation of anchoring filaments by protease released from moribund or dead cells (Papirmeister 1993). Clark and Smith (1993) showed that mustard gas treatment of HeLa cells produces a rapid stimulation of PADPRP activity, followed 2 hours later by a decline in NAD^+ levels. Several other studies provide partial support for the hypothesis, but hint that additional pathways may be involved. The hypothesis is almost fully validated in a study by Meier and Kelly (1993), in which PADPRP inhibitors prevent the mustard gas-induced losses of ATP, NAD^+, and viability in human peripheral blood lymphocytes. However, their observation that ATP levels decline before NAD^+ deviates from the expected response. Niacinamide, an inhibitor of PADPRP and a substrate for NAD synthesis reduced mustard gas-induced loss in NAD (Meier et al. 1987; Mol et al. 1989, 1991; Papirmeister et al. 1985; Smith et al. 1990) and ATP (Meier et al. 1996). 3-Aminobenzamide, an inhibitor of PADPRP but not a substrate for NAD synthesis, also reduced mustard gas-induced loss in ATP (Meier et al. 1996). Niacin, a substrate for NAD synthesis, which does not effect PADPRP, failed to prevent mustard gas-induced loss of ATP (Meier et al. 1996). These findings support the hypothesis that PADPRP plays a substantial role in mustard gas-initiated biochemical changes. Cowan et al. (1993) observed that although niacinamide-attenuated mustard gas-induced increases in protease activity *in vitro* and *in vivo*, it did not

eliminate them, suggesting that pathways other than the one involving PADPRP may contribute to the increase in protease activity. Yourick et al. (1991, 1993) noted that while pretreatment with niacinamide reduced the incidence of mustard gas-induced microvesiculation in hairless guinea pig skin, the prediction of the PADPRP hypothesis, that the loss of NAD^+ precedes tissue injury, was not upheld. Martens and Smith (1993) demonstrated that whereas mustard gas treatment of human epidermal keratinocytes (HEK) produces a dose-related depletion of NAD^+ and inhibition of glucose metabolism, preceding cell death, niacinamide did not prevent the inhibition of glycolysis, suggesting that in HEK, other energy-depleting mechanisms may be involved in mustard gas cytotoxicity. In contradiction to the hypothesis, results in rat keratinocytes exposed to mustard gas indicate that depletion of NAD is not a prerequisite for cell death (Lin et al. 1994). At doses lower than 50 µM, DNA content, viable cell number, and the proliferative capacity of the culture, as assessed by thymidine incorporation, were all reduced, whereas the total NAD level (NAD^+ plus NADH) was not changed. Also supplementing the culture with nicotinamide after exposure to mustard gas did not reverse the decrease in DNA content.

As another hypothesis for mustard gas-induced cytotoxicity, Whitfield (1987) suggested that mustard gas alkylation of glutathione (GSH) removes one of the major cellular defense mechanisms against electrophilic compounds and oxidants. Once GSH is depleted, electrophiles such as mustard gas or endogenously-generated reactive oxygen species eventually inactivate critical sulfhydryl proteins involved in calcium homeostasis and/or modify cytoskeletal elements. The subsequent inability of cells to maintain the low intracellular calcium concentration causes activation of catabolic processes leading to cell damage and death. In partial support of this hypothesis, Ray et al. (1993) demonstrated that treatment of neuroblastoma cells and HEKs with mustard gas causes depletion of GSH, raises the level of intracellular calcium, and stimulates phospholipase A_2, processes that precede and ultimately lead to membrane damage and cell death. Also, increasing cellular GSH levels decreased the toxic effects of mustard gas in human peripheral blood lymphocytes (Gross and Smith 1993).

Apoptosis may be a mechanism by which mustard gas exerts its cytotoxic effects. In keratinocytes incubated with mustard gas, p53 (a promoter of apoptosis) levels increases, while levels of bcl-2 (a suppressor of apoptosis) decreased (Rosenthal et al. 1998). The immunostaining pattern of these two markers in mustard gas treated skin excised from weanling pigs also suggests the involvement of apoptosis in cell death secondary to mustard gas exposure (Smith et al. 1997a). Thymocytes, isolated from rats, and incubated with mustard gas showed an increase in the production of DNA fragments

characteristic of apoptosis (Michaelson 2000). It is possible that mustard gas toxicity involves several independent or interacting pathways, some aspects of the various hypotheses.

Cell cycle kinetics are involved in the cytotoxic processes following mustard gas exposure. Mustard gas-induced damage at subvesicating concentrations (<50 μM) to genomic DNA in cultured HEK resulted in a dose-related reversible block at the G_2/M phase of the cell cycle (Smith et al. 1993). Okadaic acid and calyculin A, inhibitors of protein phosphatase 2A (PP2A), completely reversed the mustard gas-induced G_2/M block, whereas tautomycin, an inhibitor of protein phosphatase 1, was ineffective at reversing the block (Hart and Schlager 1997). As total cellular PP2A was not affected by mustard gas treatment; these results suggest that PP2A is involved in the G_2/M block produced by exposure of HEK to low concentrations of mustard gas. Exposure of human peripheral blood lymphocytes (PBL) to vesicating equivalent concentrations of mustard gas ($50 μM) resulted in irreversible blockage at the G1/S interface (Smith et al. 1998). DNA became terminally fragmented.

Theories have been proposed that blistering induced by mustard gas may involve cytokine production and a secondary inflammatory response (Dannenberg and Tsuruta 1993; Graham et al. 1993; Papirmeister et al. 1991). In the trachea as in the skin, mustard gas appears to preferentially damage the cells that are the most active in regeneration after aggression, basal cells located above the dermal papillae in skin (Papirmeister et al. 1991) and epithelial secretory cells in the trachea (Calvet et al. 1996). In the cell, DNA and proteins are the main targets for mustard gas alkylation; therefore, it is not unexpected that the most severe lesions effect cells with the greatest progenitorial and metabolic capacity. Eosinophils, known to produce growth factor and cytokines, were reduced in guinea pigs at 2 weeks postexposure, which may influence epithelial regeneration and result in the characteristic slow lesion repair or recovery (Calvet et al. 1996). The literature contains conflicting reports of mustard gas effects on cytokines. In cultured HEK treated with 1–100 μM mustard gas, Pu et al. (1995) observed a dose-related increase in IL-1α at 72 hours after exposure. Zhang et al. (1995) also measured an increase in IL-1α in isolated perfused porcine skin treated with mustard gas at 5 hours after exposure. In contrast, Kurt et al. (1998) who tested the effects of mustard gas on both adult and neonatal HEK, reported a dose-related decrease in IL-1α in cultured adult HEK treated with 0.5 and 1.0 mM mustard gas; however, only a minimal change in IL-1α was seen in neonatal HEK. Mustard gas applied to the mouse ear resulted in an increase in IL-6 levels at 6 and 18 hours postexposure, whereas IL-1β and TNF-α levels were unchanged (Casillas et al. 1996). Kurt et al. (1998) reported that in both neonatal and adult HEK, TNF-α was increased at 0.5 mM and decreased at 1.0 mM mustard gas, whereas IL-1β, IL-6, and IL-8 were increased at both

concentrations. While IL-1α and IL-1β share the same biological activity and recognize the same receptors on target cells, Kurt et al. (1998) suggest that the differences in the amount of each cytokine released relative to the distribution in HEK support different mechanisms of action for mustard gas with IL-1α and IL-1β. Since the decrease in IL-1β was the only cytokine of those studied with significant decreases in both neonatal and adult cell types and at both concentrations, Kurt et al. (1998) hypothesized a direct effect of mustard gas on IL-1β and indirect actions on the other cytokines.

In order to investigation possible mechanisms of blistering, urokinase, one of two mammalian activators for converting plasminogen into active plasmin, was investigated *in vitro* in cultured 3T3 fibroblasts exposed to 100 μM mustard gas (Detheux et al. 1997). Plasmin is a wide-spectrum serine protease, which is capable of degrading most extracellular and basement membrane proteins. Twenty-four hours after exposure, urokinase activity was increased 20-fold compared to control cells. The significance of this proteolytic response in the pathogenesis of blistering is not yet understood.

There have been several studies of protein alkylation by mustard gas with possible relevance to blister formation. A potential target for mustard gas alykation is uncein, an anchoring filament-associated antigen thought to play a role in maintaining the integrity of the dermal-epidermal basement membrane zone. Fractionation by SDS-PAGE and immonofluorescent staining of uncein treated with mustard gas indicated that mustard gas chemically modified uncein (Zhang et al. 1998). Male Yorkshire cross weanling pigs were exposed dermally to two vesicating doses, estimated at 21,000 and 42,000 mg-minute/m^3, of mustard gas (Smith et al. 1997a). Immunostaining of excised treated skin revealed a progressive decrease with eventual loss of expression of GB3, an antibody to basement membrane protein, laminin 5, during the time of vesiculation at both doses. Desmosomal proteins, cellular fibronectin, laminin 1, collagen IV, and collagen VII showed no change or inconsistent changes during the same period. The laminins are cystein-rich proteins with multiple thiol groups available for alkylation by mustard gas. The pattern of immunostaining for laminin 5 was consistent with electron microscopy findings showing fragmentation of anchoring filaments at the time of vesication and suggests that disruption of laminin 5 may be a factor in mustard gas-induced blistering. Laminin 5 regeneration occurs early after injury, whereas cutaneous lesions are slow-healing with no evidence of re-epithelialization at 7 days after exposure in a hairless guinea pig model. The authors suggest that residual alkylated laminin 5 and laminin 1 fragments could inhibit the functioning of the newly formed laminin 5.

DNA arrays were used to study the differential gene expression changes that occur within human epidermal keratinocytes after exposure to mustard gas (Platteborze 2000). Several genes were identified that exhibited significant transcriptional upregulation that could have roles in early mustard gas injury. Transmembrane serine protease hepsin, which is thought to be involved in cell growth, differentiation, and maintenance of morphology, was upregulated about 8-fold at 10–30 minutes after exposure. Heparin sulfate proteoglycan 2 (HSPG2) was upregulated about 13-fold at 10 minutes and about 8-fold at 30 minutes after exposure. HSPG2 is an integral component of basement membranes and is proposed to be involved in cell binding, basement membrane assembly, calcium binding, LDL metabolism, activation of serine protease inhibitors, and the anchorage of acetylcholinesterase (AChE) to the extracellular matrix of the neuromuscular junction. In addition, heparin sulfate chains carry a fixed negative charge, which is thought to participate in the selective permeability of basement membranes. Human periodic tryptophan protein 2 (yeast) homolog (PWP2H) was also significantly overexpressed, about 7-fold at 10 minutes and about 14-fold at 30 minutes. At present, little is known about the function of PWP2H. A notable absence of upregulation of nucleotide repair genes, ERCC1 (Excision Repair Cross-Complementing repair deficiency group 1) and ERCC2, and enzyme poly(ADP-ribose) polymerase (PADPRP) at 10 and 30 minutes postexposure suggests that the recognition or response of human epidermal keratinocytes to mustard gas genotoxicity is delayed, since poly(ADP-ribose) polymerase (PADPRP) activation was observed at 4 hours after exposure.

A dose-dependent inhibitory effect of mustard gas on the heat shock response was found in mononuclear human cells (Sterri 1993). The effect was fully developed at subvesicating doses and was strongly dependent on the order of the exposures to mustard gas and stress effector. Heat shock protein expression was inhibited in cells exposed to mustard gas and subsequently heat shocked, whereas cells that were heat shocked first and then exposed to mustard gas continued with the normal heat shock response. These results point to both transcriptional and translational sites of effect. The mechanistic coupling between the stress response and mustard gas remains to be understood.

Sawyer et al. (1996) examined the possibility that the toxicity of mustard gas is due to the induction or activation of nitric oxide synthase (NOS). L-nitroarginine methyl ester (L-NAME), an arginine analog inhibitor of NOS, was found to confer protection to mature primary cultures of chick embryo forebrain neurons against the toxicity of mustard gas when administered as a pretreatment or up to 3 hours postexposure. No protection was evident in immature (1-day-old) cultures. While NOS requires L-arginine as a substrate, mustard gas toxicity and L-NAME protection were independent of L-arginine

concentration. In contrast to L-NAME, L-thiocitrulline (L-TC), another arginine analog NOS inhibitor, was found to protect immature cultures of neurons against mustard gas, as well as mature cultures (Sawyer et al. 1998). L-TC increased the LC_{50} of mustard gas by approximately 800 and 1,500% with 1-hour and 24-hour pretreatments, respectively. The protection conferred by L-TC was persistent, unlike L-NAME whose protection was dependent on its continued presence, suggesting that these closely related arginine analogs act at different sites to exert their effects (Sawyer et al. 1996, 1998). A synergistic protective effect was found in mature neuron cultures pretreated with both L-NAME and L-TC (Sawyer 1998). Whereas 1-hour pretreatment with L-NAME and L-TC increased the LC_{50} of mustard gas by approximately 200 and 800%, respectively, together up to 1,500% protection was conferred in mature cultures. Based on these findings, Sawyer (1998) proposed that mustard gas initiates its toxicity rapidly through a cell-surface mediated event, that can be blocked by L-TC, followed by signal transduction into the cell with an additional event manifested several hours later. The role of NOS in mustard gas toxicity remains unclear; however, these arginine analog NOS inhibitors provide protective effects, apparently not mediated through inhibition of NOS.

A study by Zhang et al. (1995) of the protective effects of four pharmacological agents in mustard gas-treated isolated perfuse porcine skin flap (IPPSF) suggests that different mechanisms are involved in the production of mustard gas-induced dark basal cells, microvesicles, and vascular response. Reduction of mustard gas-induced dark basal cells was observed with mustard gas scavengers, sodium thiosulfate and cysteine, with niacinamide, an inhibitor of poly(adenosine diphosphoribose) polymerase (PADPRP) and a substrate for NAD synthesis, and with cycooxygenase inhibitor indomethacin. Treatments with niacinamide and indomethacin, but not sodium thiosulfate or cysteine, resulted in an inhibition of the vascular response in IPPSF exposed to mustard gas. Of the four agents, microvesicles were only partially prevented in the indomethacin-perfused IPPSF.

The toxic effects of sulfur mustard have been attributed to DNA modification with the formation of 7-hydroxyethylthioethyl guanine, 3-hydroxyethylthioethyl adenine and the cross-link, di-(2-guanin-7-yl-ethyl)sulfide. Bacterial 3-methyladenine DNA glycosylase II (Gly II) was found to releases both 3-hydroxyethylthioethyl adenine and 7-hydroxyethylthioethyl guanine from calf thymus DNA was modified with [14C]sulfur mustard, suggesting that glycosylase action may play a role in protecting cells from the toxic effects of mustard gas (Matijasevic et al. 1996).

Mustard gas was found to inhibit blood cell and tissue antioxidant enzyme activities in rats following topical application, which could impair cytoprtective defense mechanisms (Husain et al. 1996). Enzyme activities were measured at 24 hours after dermal treatment with 98 mg/mg (0.5 LD_{50}) of mustard gas. Superoxide dismutase (SOD) activity decreased significantly, 70% in white blood cells, 65% in platelets, 72% in the spleen, and 29% in brain. SOD activity in red blood cells, liver, and kidney did not change significantly following treatment. Catalase activity decreased significantly 54% in white blood cells, 23% in red blood cells and 51% in spleen, whereas activity levels in platelets, liver, kidney, and brain were not significantly altered. Glutathione peroxidase (GSH-Px) activity, as a consequence of glutathione and NADPH depletion, decreased significantly in white blood cells (42%), spleen (43%), and liver (22%). Glutathione activities in red blood cells, platelets, kidney, and brain were within 10% of control values.

A significant depletion of GSH of blood and liver was also observed in mice following dermal application of 38.7 or 77.4 mg/kg of mustard gas (Vijayaraghavan et al. 1991).

3.5.3 Animal-to-Human Extrapolations

Various models consisting of human peripheral blood lymphocytes, human skin grafts, porcine skin flaps in explant culture, human epidermal keratinocytes in culture, human eyes, hairless guinea pigs, and stratified rat epidermal cultures have been developed to study the biochemical events in sulfur mustard toxicity. As discussed in Section 3.2.1.2, short-term respiratory effects similar to those described in humans have been reported in experimental animals, which suggests that knowledge obtained regarding respiratory effects in animal models can be usefully applied to humans.

Unless people drink mustard gas directly from the container, they would not be exposed to it acutely via the oral route because it hydrolyzes quickly in water, so more data in this area is not needed. Laboratory animals with fur do not have sweat glands on most of their body and do not provide optimal models for dermal exposure.

3.6 Toxicities Mediated Through the Neuroendocrine Axis

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones. Chemicals

with this type of activity are most commonly referred to as *endocrine disruptors*. However, appropriate terminology to describe such effects remains controversial. The terminology *endocrine disruptors*, initially used by Colborn and Thomas (1992) and again by Colborn (1993), was also used in 1996 when Congress mandated the Environmental Protection Agency (EPA) to develop a screening program for "...certain substances [which] may have an effect produced by a naturally occurring estrogen, or other such endocrine effect[s]...". To meet this mandate, EPA convened a panel called the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC), which in 1998 completed its deliberations and made recommendations to EPA concerning *endocrine disruptors*. In 1999, the National Academy of Sciences released a report that referred to these same types of chemicals as *hormonally active agents*. The terminology *endocrine modulators* has also been used to convey the fact that effects caused by such chemicals may not necessarily be adverse. Many scientists agree that chemicals with the ability to disrupt or modulate the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. However, others think that endocrine-active chemicals do not pose a significant health risk, particularly in view of the fact that hormone mimics exist in the natural environment. Examples of natural hormone mimics are the isoflavinoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These chemicals are derived from plants and are similar in structure and action to endogenous estrogen. Although the public health significance and descriptive terminology of substances capable of affecting the endocrine system remains controversial, scientists agree that these chemicals may affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body responsible for maintaining homeostasis, reproduction, development, and/or behavior (EPA 1997). Stated differently, such compounds may cause toxicities that are mediated through the neuroendocrine axis. As a result, these chemicals may play a role in altering, for example, metabolic, sexual, immune, and neurobehavioral function. Such chemicals are also thought to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Hoel et al. 1992; Giwercman et al. 1993; Berger 1994).

It is possible that mustard gas modifies the feedback of endogenous hormones and, through the complex interactions of central nervous system and endocrine function regulation, behavior (i.e., libido). In a survey of 800 Iranian men who were exposed to mustard gas during the Iran-Iraq War, 279 men (34.8%) reported decreased libido, 342 (42.8%) reported no change, 6 (0.8%) reported increased libido, and 173 (21.6%) did not respond to this survey question (Pour-Jafari and Moushtaghi 1992). Of these men, 86.6% still suffered symptoms from chemical injury, namely lung and skin lesions.

There is limited evidence to suggest that mustard gas affects FSH levels and thus plays a role in reproductive function. The time course of changes in serum concentrations of total and free testosterone, LH, DS, FSH, 17 α-OH progesterone, and prolactin were studied in 16 men during the first 3 months after chemically confirmed exposure to chemical weapons containing mustard gas in 1987 during the Iran-Iraq War (Azizi et al. 1995). A group of 34 healthy unexposed men of similar age served as controls. Released from the pituitary, LH stimulates the Leydig cells to produce testosterone, while FSH stimulates the Sertoli cells to produce sperm. At 1 week after exposure, total testosterone, free testosterone, and DS were significantly lower, 57, 72, and 53%, respectively, in exposed men than in controls, while levels of the remaining hormones were comparable between groups. Total testosterone, free testosterone, and DS levels continued to decrease during the first 5 weeks after exposure. At 1 week, 4 of 16 exposed men (25%) had serum testosterone levels that were reduced by >60% below the control average; by the 5th week, the number increased to 11 (69%). DS mean values reached as low as 18% of the mean of control subjects. After the 5th week, these three hormone levels increased returning to normal levels at 12 weeks after injury. Small but significant increases in mean serum concentration of LH at the 3rd week and that of FSH and prolactin at the 5th week were measured. Normal levels of LH, FSH, and prolactin were measured at 12 weeks. FSH and LH response levels to 100 µg of gonadotropin releasing hormone (GnRH) administered intravenously during the first week after exposure, were subnormal in four of five patients. While testosterone levels in these men returned to normal 12 weeks after exposure, in a survey of 800 Iranian men who were exposed to mustard gas during the Iran-Iraq War, 279 men (34.8%) reported decreased libido, 342 (42.8%) reported no change, 6 (0.8%) reported increased libido, and 173 (21.6%) did not respond to this survey question (Pour-Jafari and Moushtaghi 1992). Of these men, 86.6% still suffered symptoms from chemical injury, namely lung and skin lesions.

In a follow-up study of 42 men, ages 18–37, injured by mustard gas during the Iran-Iraq War, serum testosterone, LH, and prolactin concentrations were normal in all men 1–3 years following exposure (Azizi et al. 1995). A comparison of the mean serum FSH concentration in 13 subjects with sperm count below 20 million and in 20 subjects with sperm counts above 60 million, revealed a nearly 2-fold increase in FSH concentration in the those with the lower sperm count; the increased FSH level was 38% above the mean FSH concentration in a group of 34 health unexposed males. Inhibition of spermatogenesis was also observed in male mice following intravenous injection of mustard gas (Graef et al. 1948). Elevated FSH has been correlated clinically with testicular failure, germinal aplasia, or hypergonadotropic hypogonadism. It appears unlikely that alteration of FSH levels is related to the

effect of mustard gas on the pituitary since LH levels were unaffected in males. A possible target is inhibin secretion by testes Sertoli cells, which suppresses pituitary FSH secretion.

Administration of mustard gas did not affect the reproductive potential of female mice because the fertility of the mice was not altered and no injurious effects were observed in the ovaries (Graef et al. 1948). Chronic (52 weeks) inhalation exposure of male rats to mustard gas (0.1 mg/m^3) was reported to produce significant dominant lethal mutation rates (a maximum of 9.4% at 12–52 weeks), but exposure of pregnant females to the same concentration for a shorter time interval did not (Rozmiarek et al. 1973).

McNamara et al. (1975) subsequently concluded from these same data that there were no differences between the control and experimental groups and no evidence of mutagenesis. The conflict between these two reports is not readily resolvable, but the fetal mortality values presented by McNamara et al. (1975) suggest at least a trend for dominant lethal effect. Complete control data and statistical analyses of the results are not presented, but percentages of fetal death at week 12 were 4.12, 4.24, and 21.05 for controls, 0.001, and 0.1 mg/m^3 exposure groups, respectively. In a dominant lethal study of mustard gas, rats were orally gavaged with 0.08, 0.2, or 0.5 mg/kg/day mustard gas 5 days/week for 10 weeks (Sasser et al. 1993). In female dominant lethality experiments, reproductive performance indicators (number of live or dead implants, resorptions, and preimplantation losses) in treated female rats mated to treated or nontreated males were not significantly different from controls. In male dominant lethality experiments (treated males were mated with untreated females), resorptions and preimplantation losses in the mid- and high-dose groups were significantly elevated. High-dose male sperm morphology data at all postexposure sampling times, 0, 5, and 12 weeks, showed a statistically significant decrease in the percentage of normal sperm. Blunthook and banana-shaped sperm heads were observed at 0, 5, and 12 weeks, whereas amorphous and short head abnormalities were observed only at 5 and 12 weeks. Overall, there was a total 2-fold increase in abnormal sperm heads in high-dose mustard gas-treated males. In summary, female fertility was not affected by these mustard gas exposures; however, a male dominant lethal effect was demonstrated at the mid and high doses of mustard gas. This lack of reproductive effect in female animals further supports the testes, rather than the pituitary, as the target organ in connection with possible mustard gas-induced alteration in FSH levels.

The time course of changes in thyroid indices, serum T3, T4, TSH, reverse T3, thyroglobulin and cortisol, plasma ACTH, and free T3 and T4 indexes (FT3I, FT4I) were studied in 13 male soldiers, ages 21–32 years, during the first 5 weeks after chemically confirmed exposure in 1987 during the Iran-Iraq

War to chemical weapons containing mustard gas (Azizi et al. 1993). A group of 34 healthy unexposed men of similar age served as controls. T4 and FT4I were not consistently affected following injury; compared to controls, significantly decreased values were measured at 1 and 5 weeks after exposure, and but values slightly above normal were measured at 3 weeks. T3 and FT3I were significantly lower (11–23%) than control at 1, 3, and 5 weeks after injury. Reverse T3 concentration in injured men was significantly higher (29%) than mean control value at 1 week, but was normal at weeks 3 and 5. TSH and thyroglobulin levels in the injured soldiers were comparable to controls during the 5 postexposure weeks. Cortisol was significantly higher (40%) than normal 1 week after exposure, within the normal range at week 3, and significantly decreased (50%) below normal at week 5. ACTH was significantly increased (57–80%) above the normal control value at 1, 3, and 5 weeks after exposure. No follow-up studies of thyroid indices were located to determine whether normal levels returned or if any chronic effects exist.

3.7 CHILDREN'S SUSCEPTIBILITY

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans, when all biological systems will have fully developed. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Relevant animal and *in vitro* models are also discussed.

Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 6.6 Exposures of Children.

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates

because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and sometimes unique enzymes may exist at particular developmental stages (Komori et al. 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns who all have a low glomerular filtration rate and have not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

Certain characteristics of the developing human may increase exposure or susceptibility, whereas others may decrease susceptibility to the same chemical. For example, although infants breathe more air per kilogram of body weight than adults breathe, this difference might be somewhat counterbalanced by their alveoli being less developed, which results in a disproportionately smaller surface area for alveolar absorption (NRC 1993).

Information on children's health effects is provided from reports of children exposed to mustard gas from air bombs during the Iran-Iraq War (Momeni and Aminjavaheri 1994). Clinical manifestations of mustard gas in the children included ocular, cutaneous, respiratory, gastrointestinal, hematological, and neurological effects similar to those seen in adults; however, the onset of symptoms in children was sooner than in adults and the severity was greater. Generally, irritant effects of mustard gas in adults are delayed by about 8 hours, whereas manifestations in children occurred as early as 4 hours after exposure. As in adults, the most severe effects were portal-of-entry effects to the eyes, skin, and respiratory tract as might be expected for a vesicant. Cough and vomiting were the first symptoms in children, but not in

adults. Genital manifestations were less frequent in children and teenagers (42%) than adults (70%); however, even within the group of children, the incidence and severity of genital lesions increased with age. Other skin lesions had no apparent age-relation. The only information regarding possible adverse developmental effects in humans is that provided by Pour-Jafari (1994b), which suggests an association between parental exposure to mustard gas and elevated rates for congenital malformations. Studies of animals exposed during pregnancy by oral gavage have indicated reduced fetal weight and reduced ossification of the vertebrae and/or sternebrae, but only at levels that were also toxic to the mother (DOA 1987b; Sasser et al. 1996a).

There is no information regarding pharmacokinetics of mustard gas in children nor it is known whether mustard gas can be stored and excreted in breast milk. There have been no direct measurements to determine whether mustard gas can cross the placenta. There is no information on whether mustard gas can be stored in maternal tissues and be mobilized during pregnancy or lactation. There is no information on the metabolism of mustard gas in children.

There are no biomarkers of exposure or effect for mustard gas that have been validated in children or in adults exposed as children. No studies were located regarding interactions of mustard gas with other chemicals in children or adults.

No information was located regarding pediatric-specific methods for reducing peak absorption following exposure to mustard gas, reducing body burden, or interfering with the mechanism of action for toxic effects. In addition, no data were located regarding whether methods for reducing toxic effects of mustard gas in adults might be contraindicated in children.

Kurt et al. (1998) report differential sensitivity related to cytokine release of cultured adult and neonatal human epidermal keratinocytes treated with mustard gas, but the significance of these findings are not known.

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s), or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to mustard gas are discussed in Section 3.8.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by mustard gas are discussed in Section 3.8.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.10 “Populations That Are Unusually Susceptible”.

3.8.1 Biomarkers Used to Identify or Quantify Exposure to Mustard Gas

It is possible that mustard gas itself may be detected in the urine if a person is exposed to very high levels. However, the need for low-level and retrospective detection of exposure has been illustrated in the attempts to clarify the causes of the significant number of postwar symptoms experienced by soldiers involved in the Persian Gulf War. Black et al. (1992a) identified, in addition to several other metabolites, thiodiglycol sulphoxide as the major urinary excretion product, and not the initial hydrolysis product thiodiglycol. In two subjects accidentally exposed to mustard gas, urine thiodiglycol sulphoxide concentrations were 20–35 times thiodyglycol concentrations (Black and Read 1995a). However, the use of thiodiglycol sulphoxide or thiodyglycol as biological markers for mustard gas poisoning is limited by their presence at low concentrations in normal human urine. Of the remaining metabolites, several are conjugates of mustard gas with N-acetylcysteine, most of which have poor mass spectrometric and/or gas chromatography properties mainly due to thermal instability (Black et al. 1991). Two closely related metabolites of mustard gas, 1,1'-sulphonylbis[2-(methylsulphinyl)ethane] and 1-methylsulphinyl-2-[2-methylthio)ethylsulphonyl]ethane, derived from the action of β-lyase on cysteine conjugates, have been detected in urine collected from Iran-Iraq War casualties of mustard gas poisoning (Black and Read 1995b). There were no background levels of these metabolites detected in human or rat urine (Black et al. 1991).

Since mustard gas is known to alkylate DNA, RNA, and proteins, initial attempts were made to detect mustard gas DNA adducts in urine, which may be released from dying cells (Somani and Babu 1989). N-alkylated purines, such as N7-hydroxyethylguanine, have been identified from enzymatic digests as active sites for mustard gas (Fidder et al. 1994; Niu et al. 1996; Somani and Babu 1989; Van der Schans et al. 1994). The N7-guanine adduct of mustard gas in DNA has been detected by immunochemical analysis in the blood of two victims of the Iran-Iraq War (Benschop et al. 1997). The complications that arise to isolate double-stranded DNA from biological samples and to make the DNA single-stranded without destruction of the mustard gas adducts result in about a 20-fold higher limit for adduct detection in DNA from human blood than in single-stranded DNA. Presently, adducts in white blood cells can be detected after exposure of human blood to sulfur mustard concentrations $2 μM (van der Schans et al. 1994). Another mustard gas metabolite, N7-(2-hydroxyethylthioethyl)-guanine, has been detected in the urine of guinea pigs exposed to mustard gas (Fidder et al. 1996a).

Van der Schans et al. (1994) synthesized N7-HETE-GMP for use as a hapten to generate monoclonal antibodies against the major adduct, N7-[2-[(hydroxyethyl)thio]ethyl]guanine (N7-HETE-Gua), formed after alkylation of DNA with mustard gas. Six stable clones producing antibody specific for mustard gas adducts were isolated from immunized mice and characterized by ELISA. These antibodies have potential in the development of a single-cell assay with immunofluorescence microscopy to quantify adduct formation in skin exposed to mustard gas.

To enable detection of low-level exposure to mustard gas, mustard gas adducts with proteins have also been explored. Mustard gas alkylates hemoglobin (Black et al. 1997a, 1997b; Fidder et al. 1996a; Noort et al. 1996, 1997) and albumin (Noort et al. 1999). In hemoglobin, the N-terminal valine, on both the α and β chains, and histidine residues were identified as key sites of interaction (Black et al. 1997a, 1997b; Noort et al. 1997). A cysteine residue of albumin was identified as a site of mustard gas alkylation (Noort et al. 1999). A procedure employing gas chromatography-mass spectrometry with modified Edman degradation has been developed for the determination of the adduct of mustard gas with the N-terminal valine residue of hemoglobin (Fidder et al. 1996a). A mass spectrometric analysis of the adduct of mustard gas with the cysteine residue of albumin, S-[2-[(hydroxyethyl)thio]ethyl]Cys-Pro-Phe, provided a detection limit for mustard gas an order of magnitude lower than the modified Edman assay for hemoglobin (Noort et al. 1999). The drawback for albumin adduct detection is the faster elimination rate. The half-life of albumin is 20–25 days versus the 120 day life span of hemoglobin. Both protein adducts have been detected in the blood of two victims of the Iran-Iraq War using the respective assay (Benschop et al. 1997; Noort et al. 1999).

3.8.2 Biomarkers Used to Characterize Effects Caused by Mustard Gas

An antibody that binds mustard gas has been developed as a tool for research and forensic detection (Lieske et al. 1992). The antibody was assessed by testing the cross-reactivity of rabbit anti-mustard gas antiserum with mustard gas and related compounds. The antiserum was inhibited to a similar degree by mustard gas, chloroethyl ethyl sulfide (CEES), and chloroethyl methyl sulfide (CEMS). Single arm nitrogen mustard produced 60% less inhibition. Thiodiglycol, the principal hydrolysis product of mustard gas, does not react with the antibody.

Myeloperoxidase (MPO) activity was measured to characterize the dose- and time-dependence of polymorphonuclear leukocyte (PMN) infiltration during the development of mustard gas lesions on the

skin of euthymic hairless guinea pigs (HGP) (Bongiovanni et al. 1993). PMNs, as assessed by MPO levels, peaked at 9 hours postexposure, irrespective of mustard gas vapor dose. At 9 hours, a maximum 20-fold increase in PMNs was measured relative to control sites. At 24 hours postexposure, MPO levels dropped to twice control levels. Because a 9-hour postexposure period coincides with epidermal detachment characterized by electron microscopy, these results suggest that PMNs participate in the HGP cutaneous inflammatory response to mustard gas dermal exposure and that MPO may be a useful biological marker for evaluating putative antivesicants.

Electrophoretic protein separation of soluble homogenate fraction of full thickness skin samples from weanling pigs exposed one time dermally to mustard gas, revealed an acute increase in an approximately 160 kDa protein band, consistent with an increase in haptoglobin (Blank et al. 1996). The amount of 160 kDa protein was related to the extent of vascular damage and may be an indicator of the severity of tissue damage. Elevated serum haptoglobin levels have been reported in rabbits exposed to hemi-mustard, but it is unknown whether any nonmustard chemicals induce a similar increase.

Pu et al. (1995) have shown that interleukin 1α (IL-1α) can be used to quantify the cytotoxicity resulting from mustard gas-induced damage to cellular DNA. A high correlation was observed between the dose-responsive increase in the amount of IL-1α in cultured human epidermal keratinocytes (HEK) after exposure to mustard gas, measured using a monoclonal antibody to human IL-1α, and the fraction of viable cells, assessed by trypan blue exclusion. The mustard gas dose-responsive increase in DNA crosslinking immediately after exposure, measured by ethidium bromide intercalation, correlated with the increase in cellular IL-1α 72 hours after exposure and was, therefore, predictive of delayed cytotoxicity in the exposed culture.

Inhalation exposure of rats to mustard gas by assay of lung lavage fluid revealed a time-dependent increase in proteolytic activity (Cowan et al. 1997). Lindsay et al. (1996) have shown that the serine protease inhibitor mafenide HCL and cysteine protease inhibitor E64 prevent the dermal-epidermal separation in human skin explants after mustard gas exposure. Compounds that reduce mustard gas-induced proteolytic activity have potential in elucidating the mechanisms of mustard gas toxicity.

3.9 INTERACTIONS WITH OTHER CHEMICALS

No data were located on the interactions of mustard gas with other toxicants likely to be found at hazardous waste sites.

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to mustard gas than will most persons exposed to the same level of mustard gas in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters result in reduced detoxification or excretion of mustard gas, or compromised function of organs affected by mustard gas. Populations who are at greater risk due to their unusually high exposure to mustard gas are discussed in Section 6.7, Populations With Potentially High Exposures.

Humans show varying degrees of sensitivity to mustard gas (Renshaw 1946; Sulzberger et al. 1947). For dermal contact, fair-skinned people are more sensitive than dark-skinned people. These reports also indicate that individuals with previous exposure are more sensitive to the dermal effects of mustard gas. It is possible that individuals with respiratory problems (asthma, emphysema, etc.) might be more sensitive to the effects of mustard gas and might suffer acceleration of their disease following exposure. Since mustard gas has been associated with lung cancer, people who smoke may be at greater risk.

Children are more susceptible to the effects of mustard gas than adults. The time of onset of mustard gas manifestations in children is shorter and the severity of the lesions is higher than in adults, possibly due to more delicate skin and epithelial tissues (Momeni and Aminjavaheri 1994). Cough and vomiting were the first symptoms in children, but not in adults. Children had higher occurrences of ocular, respiratory, and gastrointestinal effects than adults. Genital manifestations were less frequent in children and teenagers than adults, whereas other skin lesions had no apparent age-relation.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to mustard gas. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to mustard gas. When

specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. The following texts provide specific information about treatment following exposures to mustard gas:

Augerson WS, Sivak A, Marley WS. 1986. Chemical casualty treatment protocol development - treatment approaches. Vol II-IV. Cambridge, MA: Arthur D. Little, Inc.

Marrs TC, Maynard RL, Sidell FR. 1996. Chemical warfare agents. John Wiley & Sons, New York.

OPCW. 2001. Organization for the prohibition of chemical weapons, decontamination of chemical warfare agents. Http://www.opcw.nl/chemhaz/decon.htm. March 13, 2001.

SBCCOM. 2001. Material safety data sheet, mustard gas. Aberdeen Proving Ground, MD: U.S. Army Soldier and Biological Chemical Command. Http://in1.apgea.army.mil/RDA/msds/hd.htm. March 13, 2001.

U.S. Army. 1995. Treatment of chemical agent casualties and conventional military chemical injuries. Washington, DC: U.S. Department of the Army, FM 8-285. Http://www.adtdl.army.mil/cgi-bin/atdl.dll/query/info/FM+8-285. March 22, 2001.

Willems JL. 1989. Clinical management of mustard gas casualties. Annales Medicinae Militaris Belgicae, 1989, Vol 3 supp. Heymans Institute of Pharmacology, University of Ghent Medical School and Royal School of the Medical Services, Leopoldskazerne, B-900 Ghent, Belgium.

3.11.1 Reducing Peak Absorption Following Exposure

Decontamination procedures should be initiated immediately after exposure. The eyes should be washed immediately with water for at least 15 minutes, even if no symptoms are present, since it is known that ocular and dermal symptoms are delayed (Dreisbach and Robertson 1987; Goldfrank et al. 1990; Solberg et al. 1997). Of the many fluids studied for eye irrigation, none has proved more effective than tap water (Solberg et al. 1997). Contaminated clothing should be removed and the skin should be decontaminated. Decontamination should include the groin, axillae, and perineal areas. Rapid removal from skin is critical, as mustard gas penetrates skin within minutes of exposure. Topical decontamination with hypochlorite solutions was examined in euthymic hairless guinea pigs (Gold et al. 1994) and rabbits (Hobson et al. 1993). No significant wound differences were found between water only and various concentrations of hypochlorite and high concentrations were irritating to the skin; however, decontamination with a 0.5% solution is standard in many military medical systems. It has been suggested that removal of mustard gas with water alone is contra-indicated as mustard gas spreads over more skin surface and increases the area of blistering (Kumar et al. 1991). Absorbent powders such as calcium chloride may be sprinkled onto the exposed skin, allowed to absorb the mustard gas, and then

washed off with water (Solberg et al. 1997). In a study in which Fuller's earth (FE), N,N'-dichloro-bis (2,4,6-trichlorophenyl) urea (CC-2), and their various combinations (w/w ratios) were evaluated for their decontamination efficacy against mustard gas when applied on mouse skin, maximum protection was obtained with FE and CC-2 in a combination of 80:20 (w/w) (Kumar et al. 1991). The currently fielded ARMY skin decontamination kit (SDK) is the M291 SDK, which contains XE-555 resin (Amberguard 555) powder as the chief component (SBCCOM 2001).

3.11.2 Reducing Body Burden

Currently, there is no proven therapy against mustard gas. Mortality can be reduced by administration of electrolyte solutions by mouth, subcutaneously, or intraperitoneally commencing early and continuing throughout the intoxication period (Cullumbine 1947). Electrolyte replacement is needed due to losses from skin locally, and in the intestine and via saliva, vomitus, and diarrheaic stools. In mice a single dose of saline or glucose-saline (5 mg glucose/kg) offered protection to mice after topical mustard gas exposure; survival was 83% with saline treatment compared to 33% without treatment (Sugendran et al. 1994). In severely injured victims, systemic analgesics should be started after examination. Patients whose ocular injuries are limited to the conjunctiva require no additional treatment subsequent to irrigation. Corneal lesions may be detected by staining with fluorescein and examination with blue light. Treatment for injury to the cornea should include daily irrigation, mydriatics to ease the eye pain produced by spasm of the ciliary muscle and to prevent posterior iridolenticular adhesions, antibiotic drops to prevent secondary bacterial infections, local medications to control intraocular pressure, and systemic analgesics (Solberg et al. 1997). In cases of ocular injury, local anaesthetic drops should be avoided other than for ophthalmologic examination, as they are toxic to both healthy and damaged corneas. Although recommended, the use of sterile petroleum jelly to prevent the lid margins from sticking together should be delayed until after sufficient irrigation, since mustard gas will dissolve and concentrate in the jelly (Solberg et al. 1997). Ocular bandages should not be applied as they might raise the corneal temperature and increase the toxic effects (Solberg et al. 1997).

Patient care should include supportive treatment protocols for skin injury, respiratory distress, and cardiac dysrhythmias (Dreisbach and Robertson 1987; Haddad and Winchester 1990). There may be a delay of onset of toxicity in exposed individuals. Severe respiratory distress may be delayed for up to 72 hours depending on the concentration and duration of exposure (Ellenhorn and Barceloux 1988). In

cases of damage to the upper respiratory tract, antibiotic cover is recommended to prevent infection (Murray and Volans 1991).

Faster healing and less scarring have been reported when blisters were drained. While aseptic procedures are prudent for handling all bodily fluids, there are conflicting reports as to the danger of the blister fluid itself. There are no reports of mustard gas detected in blister fluid (Jakubowski et al. 2000); however, secondary blistering running proximal to an original blister, thought to be due to leaking fluid, was reported in a case of accidental exposure during destruction of mustard gas stockpiles (Bide et al. 1993).

Delayed keratitis should be treated with ocular lubricants, therapeutic lenses, and in severe cases, tarsorrhaphy (Solberg et al. 1997). Keratoplasty should be considered if there is significant opacification of the cornea accompanied by deposition of crystals and cholesterol.

In a study of the mustard gas vesication following pretreatment with topically applied agents, the most promising composition was comprised of petrolatum, sorbitan stearate, and water with either of the N-halo oxidants 1,3,4,6-tetrachloro-7,8-diphenyl-2,5-diiminoglycoluril (S-330) or 1,3-dichloro-5-5-dimethylhydantoin, and optionally, with a barrier-providing polymer such as perfluoroalkylpolyether (FOMBLIN HC/04, HC/25, or HC/R) or a polysiloxane (Kwong 1996). A topical skin protectant cream containing perfluoroalkylpolyether and polytetrafluoroethylene, ICD 2289, being developed to protect service members from exposure to chemical warfare agents, was shown to reduce the mustard gas-induced lesion area to 18% of untreated lesion area when applied as a pretreatment in rabbits (Liu et al. 1999). Canadian Reactive Skin Decontamination Lotion (RSDL), which is a 1.25 molal solution of potassium 2,3-butanedionemonoximate (KBDO) in 9:1 polyethyleneglycol monoethylether (500 nominal weight):H_2O, was shown to reduce the severity and scarring of mustard gas-induced lesions on the shaved back of guinea pigs (Bide et al. 1993). Also reported was the case of an employee who suffered minor mustard gas burns to the wrist and forearm during destruction of mustard gas stockpiles at the Canadian Defense Research Establishment Suffield (DRES). Treatment was carried out partly at DRES and partly at a local hospital. One set of burns received treatment with RSDL at DRES where it was available and another set did not as RSDL was not available at the local hospital. The blister initially without RSDL treatment burst and a series of secondary burns running proximal to the original blister formed. The RSDL treated burn was much less severe and no secondary burns formed.

An exciting new destructive absorption technology (DAT) employs highly reactive nanoparticles (RNP) to neutralize toxic substances including mustard gas. Preliminary studies indicate that RNP remain active against chemical agents when incorporated into a base cream and are compatible with skin contact (Koper et al. 1999).

Pulsed carbon dioxide (CO_2) laser debridement has been shown to be effective in clearing the epidermis of mustard gas damaged cells (Smith et al. 1997b). In weanling pigs, whose skin was exposed to mustard gas, CO_2 laser debridement of the exposed skin resulted in clearing of the cytologic atypia, reduced inflammatory infiltrate, and increased numbers of stromal cells within the papillary dermis. At 14 days postexposure, there was no significant difference between skin laser-debrided at 6, 24, or 48 hours after exposure.

Animal experiments have shown that sodium thiosulfate, N-acetyl-L-cysteine, nicotinamide, nicotinic acid, promethazine, dexamethasone, prednisone and vitamin E have decreased tissue damage, but their efficacy in humans is not known (Dabney 1991; Papirmeister et al. 1991; Vojvodic et al. 1985). Thiosulfate likely acts as a mustard scavenger, vitamin E as an antioxidant, and the corticosteroids by inhibiting lipooxygenase activity leading to synthesis of prostaglandins and leukotrienes (Borak and Sidell 1992). Application of provodine iodine (PI) ointment to the shaved back of guinea pigs, up to 10 minutes following mustard gas exposure has been shown to provide significant protection from ulceration (Wormser et al. 1997). Histopatholical evaluation of PI-treated skin showed only moderate thickening of the epidermis with slight hyperkeratosis, whereas deep epidermal ulceration involving the superficial dermis was evident without PI treatment. In a comparative study of chemical burn therapies in guinea pigs, debridement with trypsin-linked gauze (Debridase) was more effective in reducing the lesion area than surgical excision or laser ablation (Eldad et al. 1998b).

In guinea pigs injected intratracheally with mustard gas, subsequent treatment with betamethasone, a glucocorticoid, significantly increased tracheal epithelium height by about 20% and cell density compared to untreated animals (Calvert et al. 1996). Superoxide dismutase was effective in reducing the lesion area when administered before, but not after topical application of mustard gas in guinea pigs (Eldad et al. 1998a).

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

Mustard gas is thought to induce structural changes in cellular DNA, as indicated by altered dye response in flow cytometric studies (Smith et al. 1993). Reducing or preventing the ability of mustard gas to alkylate DNA and critical target molecules will reduce toxicity. Reduction of target structural changes may by possible by the use of compounds that react with or scavenge mustard gas and lower target alkylation levels. The speed at which mustard gas reacts presents a difficulty to this strategy of treatment.

Cell cycle kinetics are involved in the cytotoxic processes following mustard gas exposure. Mustard gas-induced damage at subvesicating concentrations (<50 μM) to genomic DNA in cultured HEK resulted in a dose-related reversible block at the G_2/M phase of the cell cycle (Smith et al. 1993). Okadaic acid and calyculin A, inhibitors of protein phosphatase 2A (PP2A) completely reversed the mustard gas-induced G_2/M block (Hart and Schlager 1997). Exposure of human peripheral blood lymphocytes (PBL) to vesicating equivalent concentrations of mustard gas ($50 μM) resulted in irreversible blockage at the G1/S interface (Smith et al. 1998). DNA became terminally fragmented. Compounds might be used to hold cells in a selected phase in order to permit DNA repair processes to correct the damaged DNA before normal proliferative events are allowed to proceed. Mimosine, one such inhibitor, was shown to provide limited protection against cytotoxicity of vesicating equivalenet concentrations of mustard gas in HEK and HeLa cells (Smith et al. 1998).

Reversal of secondary consequences of alkylation requires a better understanding of the biochemical pathways of toxicity and may require interventions for more than one mechanism of action. As pointed out by Papirmeister et al. (1991), this strategy would provide temporary measures, slowing down the injury process and buying time for intracellular repair processes, thereby avoiding the simultaneous necrosis of massive numbers of cells as occurs in mustard gas-induced epithelial lesions. Tissue function may remain close to normal if cell death can be spread out over a sufficiently long period of time, and dead cells are replaced through endogenous tissue repair and regeneration mechanisms.

Sawyer et al. (1996) examined the possibility that the toxicity of mustard gas is due to the induction or activation of nitric oxide synthase (NOS). L-nitroarginine methyl ester (L-NAME), an inhibitor of NOS, was found to confer protection to primary cultures of chick embryo forebrain neurons against the toxicity of mustard gas when administered as a pretreatment or up to 3 hours postexposure. While NOS requires

L-arginine as a substrate, mustard gas toxicity and L-NAME protection were independent of L-arginine concentration. Thus, the role of NOS in mustard gas toxicity remains unclear but L-NAME appears to have potential as a therapeutic drug.

Niacinamide (750 mg/kg, intraperitoneal), while not effective as a postexposure treatment, did inhibit microvesicle formation by 50% after cutaneous exposure to mustard gas in hairless guinea pigs when given as a 30-minute pretreatment (Yourick et al. 1991). In this study, when niacinamide was administered as 30-minute pretreatment, NAD^+ content in mustard gas treated skin biopsies decreased to about 40% of control levels. When niacinamide was administered at two times, both as a 30-minute pretreatment and as a 2-hour treatment, NAD^+ was maintained at control levels, but microvesicle formation was about the same as in the pretreatment only case, indicating that maintaining skin NAD^+ content did not absolutely confer protection from microvesication, nor was it a necessary factor for preventing microvesication.

Fpg protein is thought to protect cells from toxicity by removing ring-opened N-7 guanine adducts from DNA. Li et al. (1997) investigated the action of Fpg protein on the ring-opened form of the mustard gas adduct 7-hydroxyethyl-thioethylguanine (ro-HETEG). Fpg protein released ro-HETEG from DNA modified by [14C]sulfur mustard in an enzyme- and time-dependent manner, and may offer some protection against the toxic action of mustard gas.

The toxic effects of sulfur mustard have been attributed to DNA modification with the formation of 7-hydroxyethylthioethyl guanine, 3-hydroxyethylthioethyl adenine and the cross-link, di-(2-guanin-7-yl-ethyl)sulfide. Bacterial 3-methyladenine DNA glycosylase II (Gly II) was found to releases both 3-hydroxyethylthioethyl adenine and 7-hydroxyethylthioethyl guanine from calf thymus DNA was modified with [14C]sulfur mustard, suggesting that glycosylase action may play a role in protecting cells from the toxic effects of mustard gas (Matijasevic et al. 1996).

3.12 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of mustard gas is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to

assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of mustard gas.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

Acute-duration inhalation and acute- and intermediate-duration oral MRLs are derived from animal data. There is a greater need for additional chronic inhalation and dermal data over oral data as mustard gas hydrolyzes in water, and oral exposure is the least likely of the three routes. Laboratory animals with fur do not provide optimal models for dermal exposure as they do not have sweat glands on most of their body. Further exploration of relevant models including human skin grafts, porcine skin flaps in explant culture, and hairless guinea pigs is prudent to study the biochemical events in mustard gas toxicity and identify effective therapies.

Questions still remain regarding the mechanisms of toxicity of mustard gas. The database would benefit from research leading to greater understanding of the following (Papirmeister et al. 1993):

- The involvement of apoptotic and necrotic cell death processes to the cytotoxic and acute skin injurant actions of mustard gas.
- The importance of DNA repair and cell cycle traverse in skin cells that undergo apoptosis leading to lesion formation.
- The reason PADPRP inhibitors prevent losses of NAD^+, ATP, and viability in mustard gas-treated human peripheral blood lymphocytes (PBL), but fail to prevent mustard gas-induced cytotoxicity in HEK or mustard gas-induced acute skin injury.
- Any pathways, other than the PADPRP-mediated NAD^+ loss, by which mustard gas-induces inhibition of glycolysis and energy depletion in HEK.
- The mechanism(s) responsible for increasing and maintaining high levels of intracellular calcium in mustard gas exposed cells.
- Relationships between mustard gas and protein regulation in connection with vesication.
- The contribution of reactive oxygen species to mustard gas cytotoxicity.

- The role of inflammation in the development of the acute cutaneous mustard gas injury.

As our understanding of these mechanisms increases, more research is needed to identify therapeutic countermeasures.

3.12.1 Existing Information on Health Effects of Mustard Gas

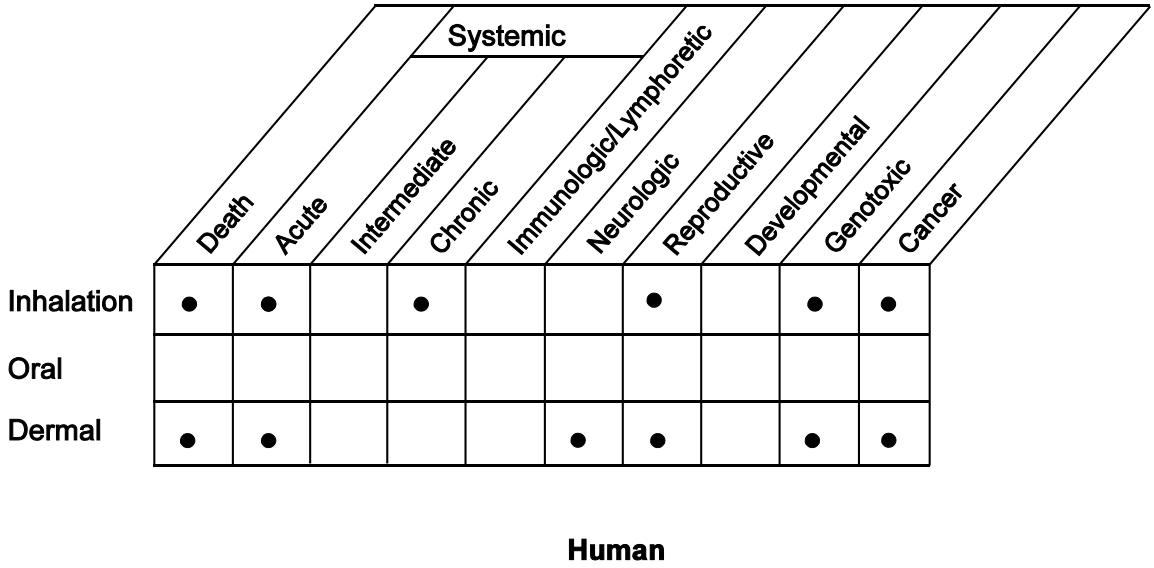
The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to mustard gas are summarized in Figure 3-4. The purpose of this figure is to illustrate the existing information concerning the health effects of mustard gas. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a "data need". A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (ATSDR 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

Data are available for humans regarding respiratory disease and cancer, and the deaths caused by these diseases following acute and chronic inhalation exposure. Very limited animal data are available regarding death, developmental and reproductive effects, and cancer following inhalation exposure. There are no data available on the toxicity of mustard gas from oral exposure in humans. Data are available for animals regarding acute and subchronic toxic effects following oral exposure. Limited data are available in humans and animals regarding skin effects from dermal exposure, and cancer in humans from dermal exposure.

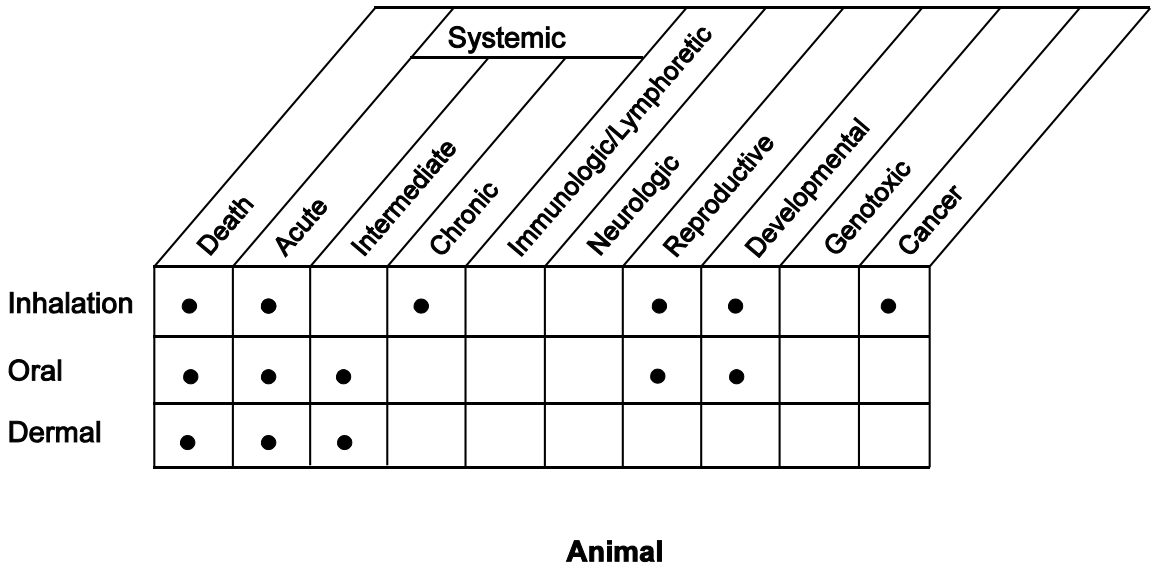
3.12.2 Identification of Data Needs

Acute-Duration Exposure. Sufficient information is available from human exposure data to identify the skin (Frank 1967; Jakubowski et al. 2000; Momeni and Aminjavaheri 1994; NRC 1985; Renshaw 1946; Sinclair 1948, 1950; Smith et al. 1919; Sulzberger et al. 1947; Wulf et al. 1985) and respiratory passages (Beebe 1960; Case and Lea 1955; Momeni and Aminjavaheri 1994; Momeni et al. 1992; Norman 1975) as target organs from acute exposure to this airborne chemical warfare agent. Data from animal studies also suggest that acute exposure to mustard gas is harmful to the gastric mucosa (DOA 1987b), skin (Chauhan et al. 1993a, 1993b, 1995; McAdams 1956; Venkateswaran et al. 1994;

Figure 3-4. Existing Information on Health Effects of Mustard Gas



Human



Animal

● Existing Studies

Vogt et al. 1984; Young 1947) and respiratory passages (Allon et al. 1993; Heston 1953b; Vijayaraghavan 1997; Winternitz and Finney 1920). Since mustard gas has been used in combat, it is known to be lethal from primary or secondary effects (Case and Lea 1955; Sinclair 1948, 1950; Somani and Babu 1989). While no human oral data are available, effects to the gastric mucosa would be expected as mustard gas is a vesicant and direct alkylating agent. Acute inhalation and oral MRLs have been derived.

Intermediate-Duration Exposure. Intermediate-duration exposure during combat has shown that mustard gas can be lethal. Wartime and occupational studies in humans have identified the skin (Bullman and Kang 2000; NRC 1985; Sinclair 1948, 1950; Wulf et al. 1985) and respiratory passages (Bullman and Kang 2000; Case and Lea 1955; Easton et al. 1988; Nishimoto et al. 1970; Somani and Babu 1989) as the target organs for mustard gas for intermediate-duration exposure. Data from animal studies also suggest that subchronic exposure to mustard gas is harmful to the gastric mucosa (Sasser et al. 1996a, 1996b). While no human oral data are available, effects to the gastric mucosa would be expected as mustard gas is a vesicant and direct alkylating agent. An intermediate-duration oral MRL has been derived. Additional studies are required in order to derive an intermediate-duration inhalation MRL. Male dominant lethal studies in animals with exposure by the inhalation and dermal routes including site of application histological examinations would provide valuable data. It seems likely that as with the oral route, the application site would be more sensitive to the effects of mustard gas than the male reproductive system; however, when considering combat exposure, the perineal area was frequently a site of application.

Chronic-Duration Exposure and Cancer. Epidemiological studies of mustard gas workers have identified the skin (Inada et al. 1978; Klehr 1984; NRC 1985) and respiratory system (Easton et al. 1988; Manning et al. 1981; Morgenstern et al. 1947; Nishimoto et al. 1970; Somani and Babu 1989; Tokuoka et al. 1986; Wada et al. 1968; Weiss and Weiss 1975; Yamada 1963; Yamakido et al. 1996) as the target organs. Chronic inhalation and oral MRLs were not derived due to the lack of quantifiable exposure data. In order to derive an MRL, additional studies with quantified exposure amounts and durations would be needed for both routes.

Factory workers who have been exposed to undetermined levels of mustard gas for a number of years have been shown to develop respiratory cancer (Easton et al. 1988; Manning et al. 1981; Morgenstern et al. 1947; Nishimoto et al. 1970; Tokuoka et al. 1986; Wada et al. 1968; Weiss and Weiss 1975; Yamada

1963; Yamakido et al. 1996). In order to develop cancer effect levels, appropriate animal studies would be necessary since there are no adequate studies currently available.

Genotoxicity. Mustard gas is known to be highly genotoxic *in vitro*, and further studies would likely not alter this conclusion (Ashby et al. 1991; Auerbach 1946; Ball and Roberts 1971/72; Capizzi et al. 1974; Fahmy and Fahmy 1971, 1972; Fan and Bernstein 1991; Ichinotsubo et al. 1977; Kircher and Brendel 1983; Lin et al. 1996a, 1996b; Ludlum et al. 1994; Ribeiro et al. 1991; Scott et al. 1974; Venitt 1968; Venkateswaran et al. 1994; Walker and Thatcher 1968).

Reproductive Toxicity. Several human and animal studies suggest that mustard gas affects the male reproductive function (Azizi et al. 1995; Graef et al. 1948; McNamara et al. 1975; Pour-Jafari and Moushtagi 1992; Rozmiarek et al. 1973; Sasser et al. 1993). Data from animal studies regarding oral exposure to mustard gas indicate that the acute-duration oral MRL derived within this profile would be protective of this system. Additional acute and chronic inhalation and oral studies are required to determine exposure levels for these routes and durations that would limit reproductive toxicity.

Developmental Toxicity. In animal studies, fetal toxicity was evidenced by reduced body weight and ossification. Data from animal studies regarding oral exposure to mustard gas indicate that the acute-duration oral MRL derived within this profile would be protective of fetal development. Additional chronic oral and acute and chronic inhalation studies are required to determine exposure levels for these routes and durations that would limit fetal toxicity.

Immunological and Lymphoreticular Toxicity. Mustard gas-induced damage to the lymph system was found in war casualties and in animals studies following inhalational, oral, or dermal exposure. Mustard gas-induced lymphoreticular toxicity does not appear to be route- or species-specific. Data from animal studies regarding inhalation and oral exposure to mustard gas indicate that the acute-duration inhalation and oral MRLs derived within this profile would be protective of the lymph system. Additional chronic inhalation and oral studies are required to determine exposure levels for these routes that would limit lymphoreticular toxicity.

Neurotoxicity. Only minimal animal data are available regarding the neurotoxicity of mustard gas (Alexander 1947; Sasser et al. 1993; Winternitz and Finney 1920). Chronic or latent pain in the exposed skin area experienced by victims of mustard gas attacks suggests that mustard gas may cause persistent damage to the afferent nerve system (Thomsen et al. 1998). This effect appears specifically related to dermal exposure and additional studies are required to determine protective exposure limits. Pharmacokinetic data are insufficient to judge the potential for mustard gas to affect this system.

Epidemiological and Human Dosimetry Studies. Two types of human epidemiology studies are available: those using men who were exposed briefly during combat in World War I (Beebe 1960; Case and Lea 1955; Norman 1975; Sinclair 1948, 1950), and those exposed for a longer period when producing mustard gas in Japanese (Nishimoto et al. 1970, 1983; Tokuoka et al. 1986; Wada et al. 1968; Inada et al. 1978; Yamada 1963; Yamakido et al. 1996), German (Weiss and Weiss 1975), British (Easton et al. 1988; Manning et al. 1981), or American factories (Bullman and Kang 2000). In these studies, exposure duration and levels were not quantified, although a relation to dose is suggested since deaths due to lung cancer increased with greater likelihood of exposure or service years in factories. Continued monitoring of mustard gas victims of the Iran-Iraq War would provide valuable toxicity information.

Biomarkers of Exposure and Effect.

Exposure. Two closely related metabolites of mustard gas not detected in normal urine, 1,1'-sulphonylbis[2-(methylsulphinyl)ethane] and 1-methylsulphinyl-2-[2-methylthio)ethyl-sulphonyl]ethane, have been detected in urine collected from Iran-Iraq War casualties of mustard gas poisoning (Black and Read 1995b; Black et al. 1991). Mustard gas has also been shown to alkylate hemoglobin (Black et al. 1997a, 1997b; Fidder et al. 1996a; Noort et al. 1996, 1997) and albumin (Noort et al. 1999). Both protein adducts have been detected in the blood of Iran-Iraq War victims (Benschop et al. 1997; Noort et al. 1999). Development and validation of standard assays for these urine metabolites and blood protein adducts would be valuable tools for retrospective detection of exposure.

Effect. Various local enzymatic activity and protein alterations have been reported in connection with mustard gas exposure, thus providing potential as biomarkers of effect. Additional research providing a further understanding of the mechanisms of mustard gas toxicity is required before assay validation.

Absorption, Distribution, Metabolism, and Excretion. There is a substantial toxicokinetic database for intravenous and intraperitoneal routes of mustard gas exposure in animals. These data indicate that it can be absorbed (Cameron et al. 1946; Cullumbine 1946, 1947; Drasch et al. 1987; Hambrook et al. 1993; Klain et al. 1991; Langenberg et al. 1998; Nagy et al. 1946; Papirmeister et al. 1984a, 1984b; Renshaw 1946; Smith et al. 1919) and is excreted in the urine (Black et al. 1992a, 1992b; Davison et al. 1961; Hambrook et al. 1992; Jakubowski et al. 2000; Maisonneuve et al. 1993; Roberts and Warwick 1963; Sandelowsky et al. 1992; Smith et al. 1958; Wils et al. 1985, 1988). Langenberg et al. (1998) detected mustard gas DNA adducts in tissues following inhalation exposure in guinea pigs. Metabolic pathways are presumed based on these data. As the route of exposure appears to be an important toxicokinetic factor, more studies would be helpful to adequately characterize the rate and extent of mustard gas absorption, distribution, and excretion via the dermal and inhalation routes.

Comparative Toxicokinetics. Data are available to indicate that the skin, respiratory tract, male reproductive system, and lymph nodes are targets in both humans and animals. Since humans do not have the fur that most laboratory animals do, and since humans have sweat glands over most of their body whereas animals do not, human responses to skin irritants such as mustard gas are different from those of animals. The hairless guinea pig model has been used to study the biochemical events in sulfur mustard toxicity. Toxicokinetic studies in animals (rats, mice, and pigs) (Black et al. 1992a, 1992b; Davison et al. 1961; Fidder et al. 1996a; Hambrook et al. 1992; Roberts and Warwick 1963; Sandelowsky et al. 1992; Smith et al. 1958), and humans (Benschop et al. 1997; Black and Read 1995b; Black et al. 1991; Jakubowski et al. 2000; Noort et al. 1999; Wils et al. 1985) indicate that the metabolites are similar across species.

Methods for Reducing Toxic Effects. Based on current concepts regarding the mechanisms of toxicity of mustard gas, compounds with known biochemical or cellular actions can be identified that may interfere with some or all of pathways of toxicity. Additional studies providing a more thorough mechanistic understanding, identification of additional pathway affectors, and validation of the efficacy of existing compounds would be valuable.

Children's Susceptibility. Besides two reports of accidental deaths of children exposed to mustard gas, clinical reports of children exposed during the Iran-Iraq War provide the only nonlethal effects data in children. The main exposure pathways for children are the same as for adults. The time of onset of mustard gas manifestations is shorter and the lesion severity greater in children than in adults, possibly

due to more delicate skin and epithelial tissues. Children's susceptibility to the effects of mustard gas is likely correlated to their understanding of the need for precautionary measures, ability to recognize exposure, and initiate decontamination.

3.12.3 Ongoing Studies

One of the major goals of future medical chemical defense research on vesicants is the search for effective prophylactic and therapeutic countermeasures. Screening programs exist for candidate antidotes.

Ongoing studies pertaining to mustard gas have been identified and are shown in Table 3-4.

Table 3-4. Ongoing Studies on Health Effects of Mustard Gas

Investigator	Affiliation	Research description	Study sponsor
Back, DD	Mainstream Engineering Corporation Rockledge, Florida	Highly destructive polymer-contained neutralizing skin protectants: Feasibility of coated topical skin protectant additives using a new class of reactive metal alloys	Army
Hendler, FJ MD, PhD	Department of Veterans Affairs Louisville, Kentucky	Effect of hazardous substances on reproductive capacity and developmental abnormalities	Department of Veterans Affairs Washington, DC
Hinshaw, DB MD	Department of Veterans Affairs Ann Arbor, Michigan	The cytoskeleton and ATP in sulfur mustard-mediated injury to endothelial cells and keratinocytes	Department of Veterans Affairs Washington, DC
Kang, HK DPH	Department of Veterans Affairs Washington, DC	Mortality follow-up of veterans exposed to mustard gas in World War II	Department of Veterans Affairs Washington, DC
Klabunde, KJ	Nantek, Inc. Manhattan, Kansas	Development of reactive topical skin protectants against sulfur mustard and nerve agents	Army
Myer, SB	Tienzyme, Inc. State College, Pennsylvania	Use of fungal peroxidases for neutralization of mustard gas	Army
Richmond, A PhD	Department of Veterans Affairs Nashville, Tennessee	The role of chemokines in wound healing and sepsis:chemical burn (sulfur mustard) model of injury	Department of Veterans Affairs Washington, DC
Sweeney, JF MD	Department of Veterans Affairs Ann Arbor, Michigan	Regulation of polymorphonuclear-leukocyte (PMN) survival and function by proinflammatory agents that are released as a consequence of sulfur mustard mediated injury	Department of Veterans Affairs Washington, DC

Source: FEDRIP 2001